## C. EIJKMAN

(August 11, 1858–November 5, 1930)

Eijkman was born August 11, 1858 at the little Dutch village of Nijkerk. He studied medicine at the University of Amsterdam, where he was an assistant to the physiologist, Professor Place.

In 1883 he went as an army surgeon to the Dutch East Indies. He soon contracted malaria and had to return to Europe for convalescence.

In 1886 he returned to Batavia. At that time a commission sent by the Dutch government to study the cause of beriberi, and if possible to indicate measures for eradicating that dangerous disease, was working in Atjeh, Sumatra, and in Java. Beriberi had been known for centuries, but only in the second half of the 19th century (as is now known, after the introduction of the steam rice mills) did it become widespread and alarming. There were prisons in Java in which imprisonment for longer than three to 4 months was equal to capital punishment; for after that time every convict died from beriberi. Also, the war against the pirates in Atjeh became impossible because the soldiers from Java, after a few months in the barracks in Atjeh, contracted beriberi. In those days the medical profession knew only two causes of disease: viz., infection and intoxication. Beriberi was a disease characterized by peripheral neuritis. Therefore the Government sent a neurologist, Professor Winkler, and a bacteriologist, Professor Pekelharing. Before they went to the tropics, these men had met Eijkman and had become so much impressed by his insight into the problems of tropical physiology that on his return to Java they accepted him as their collaborator.



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After some time Pekelharing isolated a coccus from the blood and excreta of beriberi patients, and he considered this microorganism the etiological agent of beriberi. Hence the advice of the commission: to combat beriberi by measures of disinfection. But Pekelharing was not convinced that the whole problem of beriberi was solved, and he therefore suggested to the Government that the work of the commission should be continued by Eijkman, and that a laboratory should be put at his disposal. This laboratory received the august title of: "Laboratory for Bacteriology and Pathology;" it consisted, however, of two small rooms in the Military Hospital in Batavia. Here Eijkman carried out a thorough experimental investigation of the cause of beriberi. Luckily, he chose fowls as his experimental animals, which were available in abundance at a very low cost in Batavia at that time. (Now we know that it was a great advantage that he did so, because these animals acquire avian beriberi on a diet of polished rice only; whereas in rats, for example, the other deficiencies of rice play a larger role, so that on a diet consisting of polished rice exclusively they die before they develop the signs of polyneuritis.)

Eijkman tried to inoculate the fowls with infectious material (blood, urine, et cetera) from beriberi patients; in the beginning without success. Later the inoculated fowls developed a peripheral neuritis; but so did the controls. According to Eijkman, this neuritis was quite similar to that of beriberi patients. After months the sick animals recovered and new cases of polyneuritis did not occur. What could have been the change in the circumstances of the fowls? The only possible cause that Eijkman could discern was a change in the kind of rice fed the birds. In the beginning, for economic reasons, the fowls were fed on raw rice. Later on, some weeks before the period when the fowls developed polyneuritis, they were fed the highly polished rice that was left from the tables of the patients in the hospital. Just before the recovery of the fowls, the rice was again changed to raw rice.

Now it was easy to investigate whether the kind of rice might be the cause of the polyneuritis. The experiments gave a conclusive answer: the fowls on highly polished rice developed signs of polyneuritis and could be cured on raw rice; those on raw rice remained healthy.

In the first paper in which he published these results, Eijkman wrote that it was certain that avian polyneuritis was caused by the feeding of highly milled rice, but that this did not mean that a fatal disease like beriberi in men had the same etiology. In that time "conscientious objectors" were unknown, so it was impossible to make the same experiments with men as with fowls. But Eijkman realized that perhaps this experiment was being performed on a large scale without its being realized, because the occurrence of beriberi in different prisons in Java varied so much. So Eijkman asked his friend Vorderman, who, as a health-inspector, had supervision of the prisons, to investigate in the different prisons the frequency of beriberi on the one hand, and the kind of rice that was consumed on the other hand. The results of this investigation were in accord with the outcome of Eijkman's experiments on fowls.

Therefore Eijkman stated that in men also the consumption of highly milled rice was the cause of beriberi. However, the medical profession was not convinced; even his chief, the head of the medical service in Java, wrote a long article in which he pointed out some errors of method in the work of Eijkman and Vorderman.

In 1896 Eijkman had to move back to Holland because of illness. There he was appointed Professor of Hygiene at the University of Utrecht. Eijkman was so much aware of his revolutionary views and so much impressed by this breach in the nice, simple, classical theory of nutrition that he gave to his inaugural lecture in Utrecht the title, "Simplex non very sigillum;" i.e., "Simplicity is not characteristic of truth."

This work was not his only contribution to the science of nutrition and metabolism.

At the time that Eijkman came to the tropics, every medical man was convinced that the nutrition and the metabolism of the Indians and of the white people there were quite different from those of the population in Europe and America.

The Europeans who stayed some years in the tropics looked very pale; the color of their skin was quite different from that of newcomers. This was commonly ascribed to "tropical anemia." Soon after his arrival Eijkman made a thorough examination of the number of blood cells and the content of hemoglobin and of water of the blood of both Europeans and natives.

He was astonished to find no difference at all between these constants in Java and in Europe. Thus he was obliged to destroy the myth of "tropical anemia."

Furthermore, Eijkman made a series of surveys of the nutrition and gas metabolism of Europeans in the tropics, of the natives, and especially of the soldiers. At that time it was taken for granted that metabolism in the tropics was distinctly lower than in colder regions. Even Robert Mayer, half a century ago, was led to the composition of his famous first law of thermodynamics by the observation made when he was a surgeon in the army in Surabaia (Java) that the difference in color between arterial and venous blood in the tropics was less than in Europe. Notwithstanding the soundness of his law, the observation proved afterwards to be wrong.

Again Eijkman was surprised to find that the amount of calories, and even the amount of proteins, consumed in the tropics was the same as that in Europe, if allowance were made for the much lower body weight of the natives.

The respiratory quotient appeared to be slightly higher in the natives, due to the lower fat content of their food.

Thus, Eijkman not only revolutionized the classical theory of nutrition but also existing beliefs with respect to the influence of a tropical climate on the quantitative and qualitative need for food.

After Eijkman left Java, his collaborator Grijns pursued the work on fowls to elucidate the cause of beriberi. Grijns

was the first who stated clearly that beriberi was caused by "a partial hunger;" i.e., by the deficiency of what he called "a protective factor;" he tried to isolate this substance from an extract of rice-polishings. (Funk afterwards gave this substance the name: "vitamine.")

Eijkman in Utrecht also extracted rice-polishings with acidulated water and with alcohol. In 1911 Eijkman, together with his pupil Hoogenhuyze, isolated crystals from these extracts. An injection of a solution of these crystals cured (for a short time) the polyneuritic signs in fowls; but to their disappointment, on further examination this crystalline substance proved to be a mixture of sodium and potassium chloride.

As a hygienist and bacteriologist Eijkman made many contributions to the detection of bacteriological contaminations in food and in drinking water. Best known is his famous coli-test, by which it is possible to detect whether drinking water is infected by fecal material. Eijkman found that such waters, added to a culture medium containing only glucose, pepton and salt, produced gas by incubation at 46°C.

In the beginning Eijkman's work, which was so revolutionary, did not receive much appreciation; it was only after great difficulties that he and Grijns were able to have the results of their work applied to the feeding of the people for whose diet the Government was responsible; i.e., the prisoners, the soldiers and the hospital patients.

On the other hand, Axel Holst in Norway, who was working on ship beriberi, was so much impressed by the work of Eijkman that he made the long trip to Batavia to make himself personally acquainted with the investigations that were going on there. The work of Axel Holst, as we know, led to the elucidation of the role of a new vitamin, vitamin C as it was called afterwards, in producing scurvy.

Eijkman was very modest. An American colleague once blamed him: "Eijkman, you are a bad advertiser." Nevertheless, he was honored in many ways.

McCollum dedicated his famous book on "the newer knowledge of nutrition" to Eijkman. The University of Philadelphia gave him its medal "To the most deserving."

The planters in Deli, Sumatra, who had experienced the freeing of their plantations of the scourge of beriberi through the work of Schüffner and Kuenen, as a sequence of the work of Eijkman, raised a sum of money to establish the Eijkman medal fund.

In March 1929 Eijkman received the "John Scott Medal" and in the same year the Nobel prize, jointly with Hopkins.

Eijkman was a member of the Royal Netherland Academy of Science in Amsterdam and a foreign member of the National Academy of Science in Washington.

In 1930, at the age of 72, Eijkman died in Utrecht.[1]

B. C. P. JANSEN

[1] Other sources of information are the following: *G. Grijns* (obituary) in Ned. ted. hyg. microb., 1930, *5:* 109–117, with portrait; *editorial* in the J. Am. Med. Assn., 1930, *95:* 1673; *Obituary* in Lancet, London, 1930, *2:* 1097; *G. Rijnberk* (biography) in Ned. tschr. geneesk., 1928, *72:* 2852; *Schüffner* (biography) in Arch. Schiffs Tropenhyg., 1923, *27:* 355–364; biography, pp. 217–219, and photograph, p. 505, in *The Nobel Prize Winners and the Nobel Foundation,* edited by MacCallum and Taylor, Central European Times Publishing Co., Zurich, 1938.

# NUTRITION SURVEY AND TESTS IN BATAAN, PHILIPPINES [1]

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THREE FIGURES

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## INTRODUCTION

In Bataan, the Philippines, where beriberi is endemic, an attempt is being made to reduce the prevalence of thiamine deficiency by enrichment of the major foodstuff, rice.[6] As a measure of the initial high prevalence of beriberi in this province, a clinical survey was conducted by Salcedo et al. ('48) based chiefly upon symptoms and signs of the disease. The results of the earlier survey provide a classification of subjects with beriberi of varying degrees of severity, based

[1] This study was made possible by grants from the Williams-Waterman Fund of the Research Corporation, from the Nutrition Foundation, Inc., and from the U. S. Public Health Service, Office of Research Grants and Fellowships, National Institutes of Health. Special supplies and assistance were furnished also from funds allocated to the U. S. Public Health Service Rehabilitation Program in the Philippines.

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[6] The enrichment formula includes 2 mg of thiamine and, in addition, 15 mg of niacin and 13 mg of iron per pound of rice. The estimated consumption of rice is 300 lb. per capita per year.

on the judgment of the physicians and the subjective symptoms of the people examined. Included also in the earlier report was a study of "fasting hour" urinary excretions, which proved of limited value in assessing thiamine deficiency.

The present report describes a further investigation (August, '48) of the thiamine content of body fluids, based upon biochemical measurements. The project was undertaken to evaluate in a more definite and quantitative manner the state of nutrition of a population sample and to establish a baseline of thiamine levels to be compared with later data, after the consumption of enriched rice has been practiced for a period of a year or more.

Although the study was designed primarily to secure a record of thiamine concentration in the blood samples of a small segment of the population previously classified as to beriberi symptoms, it was considered important to test concurrently for possible deficiencies of other dietary essentials and to conduct special supplementary tests.

In this paper the results of analyses are reported for thiamine and hemoglobin in blood; for protein, vitamin C, vitamin A and carotene in serum; and for thiamine in urine. Data on the effect of daily doses of thiamine on the thiamine level in the red blood cells are also given. The blood and urinary thiamine levels are compared with the degree of severity of symptoms of beriberi.

## EXPECTED DIETARY DEFICIENCIES

The previous clinical survey (Salcedo et al., '48) revealed that polished rice dominates the diet in Bataan, that two-thirds of the families use meat about once a week, that vegetables are eaten sparingly, and that fish is consumed by 90% of the population 6 or 7 times a week.

Dietary studies on 559 families of laborers in rural parts of the Philippines by Miller, Louis and Yanazawa ('46) showed the following caloric distribution (Concepcion's recommendations ['40] for better distribution are given in parentheses): cereals, mainly rice, 83.4% (50%); animal pro-

tein foods, 6.6% (10%); milk and dairy products, 0.1% (10%); fruits, vegetables and legumes, 7.8% (17%); and sugar, candy and fats, 2% (13%). Typical diets in the rural Philippines are therefore likely to be deficient in fat, animal protein, calcium, iron, carotene, and vitamins A, B complex, and C. The extremely high consumption of refined rice insures a low level of thiamine in the diet even though clinical symptoms of beriberi may not be apparent. The predisposition of pregnant women, nursing mothers, and infants to beriberi shows clearly the results of such a diet.

## METHODS OF SAMPLING

In the clinical survey the classifications of Metcoff et al. ('45) for nutritional deficiencies were applied to beriberi. Three classes were designated, as follows: (1) frank or apparent beriberi, when the subject exhibited either three signs or two signs and one or more symptoms; (2) suggestive or suspected beriberi, when the subject had either two signs or one sign and one or more symptoms; and (3) doubtful beriberi, when the subject showed one sign or when the signs were less definite.

On the basis of their symptoms, 151 individuals classified as having beriberi were chosen from Orani and surrounding villages in Bataan. All were young adult women, of whom 54 were pregnant and 54 were lactating. In addition, 25 women and 26 men were classified as "normal," but should have been more accurately termed "non-symptomatic," since their diets, as previously described (Salcedo et al., '48), were relatively low in thiamine and several other nutrients. Blood samples were taken from 202 persons.

Most of the test persons had been examined for symptoms of beriberi within two weeks before samples were taken for biochemical measurement. Forty-two, classified during the previous year, are referred to separately in the manuscript.

Blood was taken from a free-flowing finger puncture after the first drop had been discarded. The samples for hemoglobin were dropped into a gradient tube, and duplicate

samples for thiamine analyses were pipetted directly into measured volumes of 5% trichloroacetic acid and mixed at once. Samples for serum were collected in capillary tubes and were stored in a thermos jug containing ice until the collections for one day were completed. The serum was separated and serum protein measurements were made. Aliquots of serum for ascorbic acid were pipetted into measured volumes of 5% trichloroacetic acid; these samples and those of the remaining serum were frozen in dry ice. Aliquots of blood thiamine filtrates were frozen in dry ice until ready for analysis in New York.

Samples for red blood cell thiamine were taken from 6 persons at the beginning and end (the 8th day) of a 7-day realimentation period during which each person took one 10-mg thiamine tablet a day. One-milliliter samples of blood were taken by veni-puncture and delivered at once into 5 ml of 1.7% potassium oxalate. After the cells had settled at room temperature the supernatant liquid and top layer of cells were drawn off. The remaining cells were packed by centrifuging for an hour. Again the supernatant liquid and top layer of cells were drawn off. A 40 $mm^3$ aliquot was then pipetted with rinsing into 1 ml of 1% sodium chloride. The samples were then frozen in dry ice until analyzed.

To avoid the collection of urine over a measured time period, both thiamine and creatinine were determined in the same random urine specimen and thiamine was calculated per gram of creatinine (Adamson et al., '45). Urine samples were collected from 20 non-symptomatic persons and from 20 individuals in the group classified as having beriberi.

## METHODS OF ANALYSIS

Analyses were made by the following methods: hemoglobin and serum protein, Lowry and Hunter ('45); calculations of hemoglobin concentration and hematocrit, Phillips et al. ('43); ascorbic acid, Lowry et al. ('45); vitamin A and carotene, Bessey et al. ('46); blood thiamine, Burch, Bessey and Lowry (in preparation); urinary thiamine and creatinine,

Lowry, Bessey and Davis (in preparation). Urinary thiamine values were calculated in relation to 1 gm of creatinine, roughly the quantity excreted per day by a small adult.

TABLE 1

*Thiamine levels in blood and red blood cells of Filipinos in Bataan*

(Results are given as the average ± the standard error of the mean)

| CLASS OF BERIBERI | NO. OF PERSONS | THIAMINE | |
|---|---|---|---|
| | | Blood [1] | R.B.C.[2] |
| | | *μg/100 ml* | *μg/100 ml* |
| Frank | 20 | 3.2 ± 0.20 | 8.9 ± 0.61 |
| Suspected | 52 | 3.4 ± 0.14 | 9.0 ± 0.36 |
| Doubtful | 68 | 3.7 ± 0.12 | 9.3 ± 0.29 |
| Non-symptomatic | 50 | 4.0 ± 0.18 | 9.4 ± 0.41 |
| Frank and suspected | 72 | 3.4 ± 0.11 | 9.0 ± 0.31 |
| Doubtful and non-symptomatic | 118 | 3.8 ± 0.10 | 9.4 ± 0.25 |
| All persons | 190 | 3.6 | 9.2 |

[1] If only the beriberi cases examined two weeks prior to the biochemical sampling are included, the blood thiamine averages are: 3.1 ± 0.28 μg per 100 ml for 14 frank cases; 3.3 ± 0.13 μg per 100 ml for 46 suspected cases; and 3.7 ± 0.14 μg per 100 ml for 44 doubtful cases.

[2] The thiamine in red blood cells was calculated from data on thiamine in whole blood and the hematocrit, assuming the plasma contained 0.1 the amount in red cells.

## RESULTS

### *Blood thiamine*

The average values of blood thiamine levels for the various test groups (table 1) were progressively lower with increasing severity of the recognized symptoms and signs of beriberi. The average thiamine value for the total number of persons tested, 3.6 μg per 100 ml, as well as the average of the non-symptomatic persons, 4.0 μg, is distinctly lower than the average value, 6.0 μg per 100 ml, found by the same method in a small number of well-nourished adults in New York. Seven samples collected from one of the authors (H. B.) during the testing period in Bataan gave an average value of 5.9 μg per 100 ml.

Although observed differences in the means of blood thiamine levels among the various classes of beriberi are small (table 1), statistical study reveals that differences between the combined doubtful and non-symptomatic groups and the combined frank and suspected groups are significant ($P<$

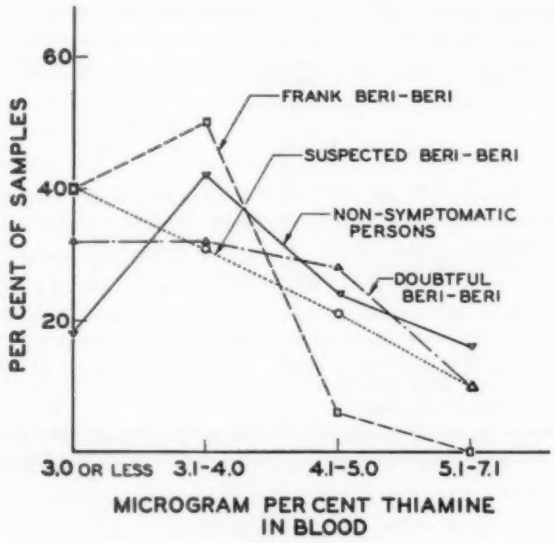


Fig. 1 The percentage of persons in various classifications of beriberi showing specified levels of thiamine in the blood.

0.01); that differences between the frank and non-symptomatic groups and between the suspected and non-symptomatic groups are significant ($P<0.01$); and that differences between the frank and suspected classes ($P<0.05$) and between the doubtful and non-symptomatic classes ($P<0.2$) are not significant. Inclusion of the 42 persons who were classified as to their symptoms more than two weeks previous to the sampling for chemical tests did not alter the interpretation of significant differences between the groups mentioned above. This is not surprising, since no changes in the avail-

ability or processing of foods or in the economic conditions of the peninsula had been inaugurated during the intervening period. Consequently these cases are included in the total number.

Figure 1, giving the percentage falling within specified levels of blood thiamine, illustrates the small proportion of Bataan residents who came within the range of 4.8 to 8.1 μg per 100 ml found by this method in individuals known to have received a good diet for a long period. Graphic distribution also shows that 40% of all frank and suggestive beriberi cases had thiamine values of below 3 μg per 100 ml, whereas only 20% of the non-symptomatic and about 30% of the doubtful groups fell below 3. Of all frank cases, 90% were below 4 μg per 100 ml, whereas of the non-symptomatic group 62% fell below 4. It is evident that in a population where beriberi is endemic, low levels of blood thiamine are prevalent among people who show no apparent clinical symptoms of deficiency.

*Thiamine in red blood cells*

Average concentrations of thiamine in red blood cells (table 1), calculated from data on whole blood thiamine and hematocrit values, show small decrements with increasing severity of beriberi symptoms. The differences between the groups are not statistically significant. The average values fall very close to the average, 9.4 μg per 100 ml, found by direct determination of thiamine in the red cells of well-nourished individuals. However, the calculated levels include the contribution due to white blood cells and platelets and are corrected for an assumed plasma level of one-tenth the level in red blood cells. The results of this indirect procedure might therefore differ from those of direct analyses on red blood cells.

Realimentation with 10 mg of thiamine per day for 7 days increased the thiamine content of the red blood cells of 6 beriberi cases (table 2). Although the numbers are too few to have statistical significance, it is worth noting that the

initial levels of thiamine in the red blood cells of suspected cases were lower than those of doubtful cases. The increase in the doubtful cases was considerably less than that of the suspected cases. The average thiamine content of the red blood cells increased 35% above the original level.

### *Blood and urinary thiamine in 40 persons*

Twenty non-symptomatic persons had an average urinary excretion of 71.4 μg thiamine per gram of creatinine (table 3). Of these 45% were below 50 μg and 85% were below 100 μg. Only one person exhibited an excretion value within the normal range of 150 to 300 μg per gram of creatinine (Adamson et al., '45). The average blood level in the same subjects was 3.9 μg per 100 ml; 55% of those tested were below 4. The average red blood cell thiamine was 9.2 μg per 100 ml of cells, with 60% of the persons above the average and 20% below 7. These results emphasize the low levels of thiamine in both the blood and the urine of residents in this area. The data furnish evidence of low thiamine intake among the non-symptomatic group and may represent levels

TABLE 2

*Thiamine in red blood cells of Filipinos after realimentation with 10 mg of thiamine daily for 7 days*

| CLASS OF BERIBERI | THIAMINE IN RED BLOOD CELLS | | |
|---|---|---|---|
| | Initial | After dosage | Increase |
| | *μg/100 ml* | *μg/100 ml* | *μg/100 ml* |
| Doubtful cases | | | |
| 1 | 8.3 | 9.6 | + 1.3 |
| 2 | 6.8 | 7.6 | + 0.8 |
| 3 | 10.3 | 11.1 | + 0.8 |
| Ave. | 8.5 | 9.4 | + 0.9 |
| Suspected cases | | | |
| 4 | 7.0 | 8.8 | + 1.8 |
| 5 | 6.5 | 12.9 | + 6.4 |
| 6 | 5.9 | 10.9 | + 5.0 |
| Ave. | 6.5 | 10.9 | + 4.4 |
| Ave. 6 cases | 7.5 | 10.1 | + 2.6 |

where, on the basis of our present knowledge, signs of beriberi might be expected to occur under conditions of stress. Williams et al. ('42) observed mild symptoms of thiamine deficiency when urinary excretion fell below 50 μg per day.

The average urinary excretion of thiamine for 20 test persons showing symptoms of beriberi was 47.3 μg per gram of creatinine. Of these, 65% were below 50 μg and 95% below 100 μg. None were in the normal range. For the same group,

TABLE 3

*Blood and urinary thiamine levels of Filipinos in Bataan*

(The results are given as the average ± the standard error of the mean)

| CLASS OF BERIBERI | NO. OF PERSONS | BLOOD THIAMINE [1] | R.B.C. THIAMINE [2] | URINARY THIAMINE [3] |
|---|---|---|---|---|
| | | *μg/100 ml* | *μg/100 ml* | *μg/gm creatinine* |
| Frank and suspected | 20 | 3.1 ± 0.26 | 8.0 ± 0.71 | 47.3 ± 3.1 |
| Non-symptomatic | 20 | 3.9 ± 0.27 | 9.2 ± 0.58 | 71.4 ± 4.4 |

[1] Distribution of the frank and suspected cases at various levels of blood thiamine was as follows: 3.0 μg per 100 ml or less, 55% of the cases; 3.1–4.0, 30%; 4.1–5.0, 15%. Distribution of the non-symptomatic persons was: 3.0 or less, 25% 3.1–4.0, 30%; 4.1–5.0, 30%; and above 5.1, 15%.

[2] These values for red blood cell thiamine were calculated as for table 1.

[3] Distribution of the frank and suspected cases at various levels of urinary thiamine per gram of creatinine was as follows: 25 μg or less, 30% of the cases; 25–50, 40%; 51–100, 30%; above 100, 0. Distribution of the non-symptomatic persons was: 25 μg or less, 20% of the persons; 25–50, 25%; 51–100, 40%; above 100, 15%.

the average blood thiamine was 3.1 μg per 100 ml and 85% exhibited blood levels below 4. The average red blood cell thiamine was 8.0 μg per 100 ml and 45% of the persons were below 7. It is apparent that a considerably larger portion of the symptomatic group than of the non-symptomatic group fell in the low thiamine range in both urinary excretion and blood levels. In individual cases, however, the urinary excretion did not always parallel the blood levels.

### *Hemoglobin*

The average hemoglobin for non-symptomatic Filipino men (table 4), 12.5 gm per 100 ml, and the range of values, 8.9 to 15.0 gm per 100 ml, was lower by a considerable margin than the normal range, 14.3 to 17.4, for American men as reported by Phillips et al. ('43).

The average hemoglobin value of each group of women (table 4), as well as the average of the total number, was considerably less than the lowest figure of the normal range,

TABLE 4

*Hemoglobin, serum protein, and hematocrit values of Filipinos in Bataan*

(Results are given as the average ± the standard error of the mean)

| CLASS OF BERIBERI | NO. AND SEX OF PERSONS | HEMOGLOBIN | HEMATOCRIT | SERUM PROTEIN |
|---|---|---|---|---|
| | | *gm/100 ml* | *% R.B.C.*[1] | *gm/100 ml* |
| Frank | 21 F | 9.6 ± 0.45 | 28.6 ± 1.31 | 6.55 ± 0.16 |
| Suspected | 57 F | 10.4 ± 0.21 | 30.8 ± 0.62 | 6.63 ± 0.10 |
| Doubtful | 72 F | 11.2 ± 0.19 | 33.1 ± 0.56 | 6.74 ± 0.08 |
| Non-symptomatic | 25 F | 11.5 ± 0.15 | 34.0 ± 0.44 | 6.92 ± 0.07 |
| | 25 M | 12.5 ± 0.29 | 37.1 ± 0.89 | 6.87 ± 0.08 |
| Frank and suspected | 78 F | 10.2 ± 0.20 | 30.2 ± 0.58 | 6.60 ± 0.09 |
| Doubtful and non-symptomatic | 123 [2] | 11.4 ± 0.14 | 34.0 ± 0.42 | 6.79 ± 0.05 |

[1] Red blood cells.

[2] The hemoglobin of the 97 women of this group was 11.3 ± 0.13 gm per 100 ml; the hematocrit, 33.3 ± 0.44%.

12.8 to 15.7 gm per 100 ml, for American women as reported by Phillips et al. ('43). Only a small proportion of the Filipino women fell within this range: 5% of women in the frank class, 9% in the suggestive, 14% in the doubtful, and 4% in the non-symptomatic.

A trend downward in the average hemoglobin levels of women paralleled in general the increasing severity of beriberi symptoms. The average of the doubtful class, however, fell very close to the average of the non-symptomatic women.

Statistically significant differences in the hemoglobin levels of women were found between the frank and non-symptomatic

groups ($P<0.001$); between the suspected and non-symptomatic groups ($P<0.001$); between the frank and doubtful and the suspected and doubtful groups ($P<0.01$); and between the combined frank and suspected and the combined doubtful and non-symptomatic groups ($P<0.001$). Differences were not significant between the frank and suspected

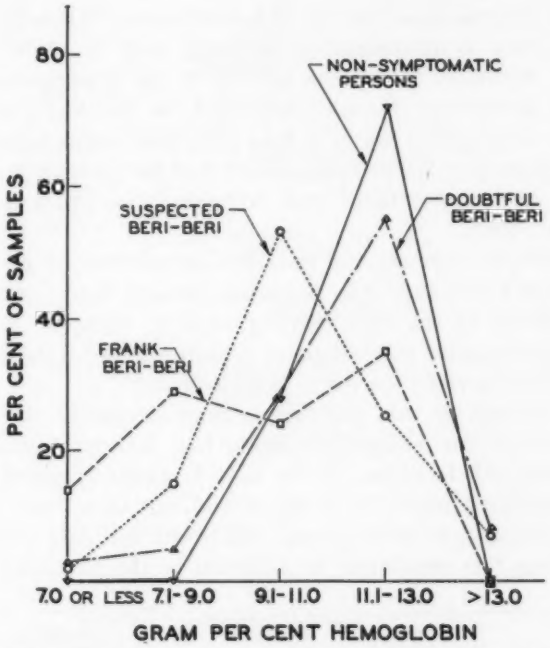


Fig. 2 The percentage of women in various classifications of beriberi showing specified levels of hemoglobin.

or between the doubtful and non-symptomatic groups. Similar significant differences were found in the hematocrit values of the various groups.

Data on the hemoglobin of the women in the various classes of beriberi are presented (fig. 2) to show the percentage of samples at specified levels. The distribution of samples shows

that 66% of frank cases, 70% of suspected, 36% of doubtful, and 16% of non-symptomatic fell below 11 gm per 100 ml. Anemia was prevalent in all groups, particularly in frank and suspected beriberi groups. Twenty-eight women (16%) were below 9 gm hemoglobin per 100 ml. These low hemoglobin values emphasize the existence of a serious nutritional and medical problem.

These findings confirm the observations of Stransky ('49a) that anemia is prevalent in Bataan, both in children and adults. Stranksy found the anemia to be hypochromic, due to iron deficiency. He also reported, on the basis of blood studies of pregnant women, that 95% had hemoglobin levels below 10 gm per 100 ml, and stated that the anemia is characterized by microcytosis and hypochromia (Stranksy and Aragon, '49b).

No data to indicate the possible association of pregnancy and anemia are available from the present study other than those shown in the first three groups in table 6. Even the non-symptomatic, non-pregnant group showed a hemoglobin level below normal for other populations.

The anemia of this population may be partly responsible for some of the subjective signs, i.e., fatigue and nervous symptoms, of beriberi. It is not, however, limited to the symptomatic groups. It is also found, but to a lesser degree, in the non-symptomatic group. The data indicate a need for correcting this condition, in addition to the thiamine status.

### *Serum protein*

Serum protein levels (table 4) for the various groups of test persons revealed no significant differences between the groups. Of the total number of persons tested, only 9% had serum protein below 6 gm per 100 ml, whereas 75% were above 6.4. Data are presented in figure 3 on the percentage of persons in various classes of beriberi showing different levels of serum protein (Bessey and Lowry, '47). In the frank beriberi group a larger percentage of persons had low serum protein levels than in the other groups. Only

one-half of them were above 6.4 gm per 100 ml. The mean serum protein, 6.73 gm per 100 ml, for the 202 persons tested agrees closely with the value 6.66 gm per 100 ml found in Filipinos by Bona and Concepcion ('40).

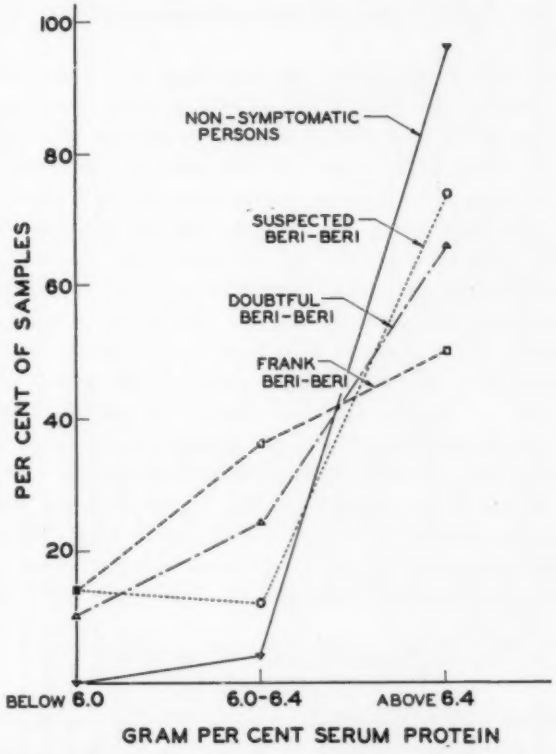


Fig. 3 The percentage of persons in various classifications of beriberi showing specified levels of serum protein.

### *Serum vitamin C, vitamin A, and carotene*

The serum levels of vitamins A and C and carotene found in the Bataan group are compared in table 5 with those found in New York high school students (Bessey and Lowry, '47)

TABLE 5

*Comparative per cent distribution of subjects having specified levels of serum ascorbic acid, vitamin A, and carotene in Bataan, Newfoundland, and New York high school surveys*

| | NO. OF PERSONS | SERUM ASCORBIC ACID [1] *mg per 100 ml* | | | | SERUM VITAMIN A [2] *µg per 100 ml* | | | | SERUM CAROTENE [3] *µg per 100 ml* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Below 0.4 | 0.4–0.69 | 0.7–1.09 | 1.1 and above | Below 20 | 20–29 | 30–49 | 50 and above | Below 75 | 75–124 | 125–199 | 200 and above |
| | | % | % | % | % | % | % | % | % | % | % | % | % |
| Bataan, Philippines | 202 [4] | 53 | 30 | 13 | 4 | 2 | 9 | 66 | 23 | 89 | 8 | 3 | 0 |
| New York school C | [5] | 48 | 25 | 17 | 10 | 0 | 4 | 83 | 13 | 16 | 46 | 32 | 6 |
| New York school G | [5] | 4 | 20 | 31 | 45 | 0 | 1 | 54 | 45 | 5 | 29 | 50 | 16 |
| Eight New York high schools | [5] | 29 | 26 | 24 | 21 | 1 | 4 | 74 | 21 | 9 | 40 | 42 | 9 |
| Newfoundland | 370 | 59 | 17 | .. | .. | 48 | 26 | .. | .. | 29 [6] | 55 [6] | .. | .. |

[1] The average serum ascorbic acid value was 0.46 ± 0.014 mg per 100 ml.

[2] The average serum vitamin A value was 47.3 ± 1.3 µg per 100 ml.

[3] The average serum carotene value was 42.0 ± 0.6 µg per 100 ml.

[4] Vitamin A and carotene figures include 193 persons.

[5] Between 120 and 150 students were tested in each school.

[6] These figures represent the per cent of persons below 50 µg and between 50 and 100 µg carotene per 100 ml serum.

and those found in a sample of the Newfoundland population (Adamson et al., '45). High school G represents a school of superior general nutritive status; school C, one of less favorable nutritive status. The Newfoundland group represents a poorly nourished population. These data were obtained by the same micro methods used in the survey in Bataan.

Low ascorbic acid and carotene levels in serum (table 5) indicate a low intake of fresh fruits and vegetables. These findings are in accord with dietary data on the Bataan population found by Salcedo et al. ('48). The average serum ascorbic acid, 0.46 mg per 100 ml, was about the same as that reported for the poorly nourished Newfoundland group, 0.49 mg per 100 ml, whereas the average serum carotene, 47 μg per 100 ml, was considerably lower than the Newfoundland average of 73 μg per 100 ml. Both values are less than one-third of those found in well-nourished New York high school students, for whom serum ascorbic acid averaged 1.66 mg per 100 ml and serum carotene 146 μg.

The average serum vitamin A for the Bataan survey, 42 μg per 100 ml, compared favorably with that of the well-nourished New York group, 37 μg per 100 ml. It is nearly twice the average, 22 μg, found in the Newfoundland samples. Actual sources of vitamin A in the Filipino diet have not been sufficiently investigated to account for the serum level.

### *Blood levels of nutrients in pregnant and lactating groups*

Women in the various classes of beriberi were subdivided into those who were pregnant, lactating, and neither pregnant nor lactating. Levels of blood thiamine and hemoglobin, thiamine in red cells, and levels of serum protein and ascorbic acid are shown for each group in table 6. The subdivision resulted in groups generally too small to show statistically significant differences between classes of beriberi. Unfortunately, there were no pregnant and only two lactating women in the non-symptomatic group. However, some trends in the data are of interest. Average values for serum protein, hemo-

TABLE 6

*Levels of thiamine and other nutrients in the blood of pregnant, lactating, and non-pregnant or lactating women in various classes of beriberi*

| CLASSIFICATION OF BERIBERI | NUMBER OF WOMEN | BLOOD THIAMINE | R.B.C.[1] THIAMINE | HEMOGLOBIN | SERUM PROTEIN | SERUM VITAMIN C |
|---|---|---|---|---|---|---|
| | | *μg/100 ml* | *μg/100 ml* | *gm/100 ml* | *gm/100 ml* | *μg/100 ml* |
| Frank | | | | | | |
| a[2] | 9 | 3.0 ± 0.31 | 8.6 ± 1.4 | 9.5 ± 0.6 | 6.09 ± 0.15 | 0.58 ± 0.11 |
| b | 5 | 3.7 ± 0.39 | 9.9 ± 1.0 | 10.5 ± 0.5 | 6.77 ± 0.25 | 0.27 ± 0.04 |
| c | 7 | 3.0 ± 0.36 | 8.7 ± 0.9 | 9.0 ± 1.0 | 6.98 ± 0.22 | 0.39 ± 0.06 |
| Suspected or suggestive | | | | | | |
| a | 21 | 2.9 ± 0.22 | 8.8 ± 0.7 | 9.7 ± 0.3 | 6.32 ± 0.10 | 0.44 ± 0.05 |
| b | 22 | 3.7 ± 0.22 | 9.5 ± 0.5 | 11.1 ± 0.3 | 6.75 ± 0.14 | 0.35 ± 0.04 |
| c | 14 | 3.3 ± 0.24 | 8.4 ± 0.6 | 10.5 ± 0.4 | 6.92 ± 0.13 | 0.56 ± 0.10 |
| Doubtful | | | | | | |
| a | 24 | 3.5 ± 0.24 | 9.4 ± 0.6 | 10.5 ± 0.4 | 6.56 ± 0.16 | 0.43 ± 0.05 |
| b | 27 | 3.8 ± 0.17 | 9.5 ± 0.5 | 11.7 ± 0.3 | 6.92 ± 0.16 | 0.33 ± 0.04 |
| c | 21 | 3.6 ± 0.19 | 9.0 ± 0.5 | 11.4 ± 0.4 | 6.74 ± 0.16 | 0.41 ± 0.04 |
| Non-symptomatic | | | | | | |
| b | 2 | 4.5 | .. | 11.5 | 6.94 | 0.53 |
| c | 23 | 3.7 ± 0.21 | 9.1 ± 0.4 | 11.5 ± 0.2 | 6.93 ± 0.08 | 0.85 ± 0.08 |

[1] Red blood cells.
[2] Group a were pregnant, b lactating, and c neither pregnant nor lactating women.

globin, and blood thiamine in the pregnant groups were lower as the symptoms of beriberi increased in severity. Even the average red cell thiamine of the pregnant women in the frank and suspected groups was lower than that of those in the doubtful group.

The serum vitamin C was low in all groups except the non-symptomatic group. The lactating women, however, showed the lowest serum concentrations, illustrating the added demand for vitamin C during lactation.

## DISCUSSION

This study was concerned primarily with the chemical determination of levels of thiamine and other nutrients in blood and serum as a measure of dietary intakes and as an indication of bodily stores in a sample of the Bataan population. The clinical classification of the test persons as to beriberi symptoms made it possible to relate the findings on thiamine in blood to the severity of the symptoms if sufficiently large groups were compared. Since the blood levels and clinical ratings measured different, but in some ways related, phenomena, it is not surprising that in individual instances the low levels in blood did not parallel the severity of the symptoms of beriberi. The blood level primarily reflects dietary intake, whereas the appearance of tissue changes is indicative of protracted deficiency. The many factors and circumstances which affect the blood levels of nutrients and clinical diagnoses for deficiencies are reviewed in the Bulletin of the National Research Council, no. 117 (Committee on Nutrition Surveys, '49). For thiamine they are less well established than for some other nutrients, i.e., vitamin C and vitamin A. The delineation of levels of blood and urinary thiamine indicative of beriberi remains open for further investigation. The biochemical data on the thiamine in blood reported here suggest that the frank and suspected cases of beriberi belong in one category and that the doubtful and non-symptomatic belong in another.

The simultaneous occurrence of low levels of hemoglobin, serum protein, and thiamine in the blood of the pregnant women examined suggests a relationship among them which may be pertinent to the appearance of more severe symptoms of beriberi, since other persons on the same diet did not exhibit these symptoms. Thus the appearance of symptoms of beriberi may be, in part, a result of stress conditions, e.g., pregnancy, in addition to low thiamine levels. However, it is evident that some of the symptoms commonly associated with disturbances of pregnancy were entered in the record (Salcedo et al., '48) as beriberi symptoms. Hence, there may have been a tendency to introduce a fortuitous relation on the basis of overlapping symptoms. More complete and extensive study would be necessary definitely to establish these relationships.

No clinical classification was attempted for symptoms of other deficiencies which the low blood or serum levels indicated may have existed.

## SUMMARY

A nutrition survey by microchemical methods has been made in Bataan on 202 persons. Three-fourths of the group had been classified by an earlier clinical survey on the basis of severity of beriberi symptoms. One-fourth were regarded as normal individuals exhibiting no symptoms of beriberi. Initial blood thiamine levels were established as a baseline for measuring improvement after the population has consumed enriched rice over a period of time.

Evidence of nutritional deficiency due to lack of thiamine was found in the low blood and low urinary thiamine levels of the population sample. The average blood thiamine of all persons was 3.6 μg per 100 ml of blood; of the non-symptomatic group, 4.0 μg. A progressive decrease in the average value for each group occurred with the increasing severity of signs and symptoms of beriberi. Statistically significant differences in blood thiamine were found between the non-symptomatic group and the frank and suspected beriberi

groups. Similarly significant differences were evident between the combined frank and suspected classes and the combined non-symptomatic and doubtful groups. The calculated red blood cell thiamine levels did not exhibit differences between these groups. The serum protein and hemoglobin levels and the hematocrit values showed progressive decrease with increasing severity of the signs and symptoms of beriberi. Concurrent low levels in blood thiamine, serum protein, and hemoglobin suggest an interrelation among these factors which may be pertinent to the appearance of symptoms of beriberi.

Evidence of multiple dietary deficiencies in Bataan was found in the levels of other nutrients in blood or serum. Widespread anemia was evident from the low hemoglobin and hematocrit values, probably as a result of chronic deficiencies of iron and several members of the B complex.

Serum protein levels fell in the lower range of normal. The lowest levels occurred in the frank beriberi group of pregnant women.

Serum vitamin A levels were normal.

Serum vitamin C and carotene concentrations were very low, apparently reflecting a low consumption of fresh fruits and vegetables. The lowest vitamin C levels were found in the group of lactating women.

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# OBSERVATIONS ON AN UNKNOWN DIETARY FACTOR ESSENTIAL FOR HUMAN GROWTH [1]

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ELEVEN FIGURES

(Received for publication June 13, 1949)

Knowledge of human dietary requirements has been derived in large part from studies of laboratory animals. However, numerous discrepancies in the requirements of various species are known to exist and in the last analysis human dietary needs must be established by direct studies of man. In most human studies natural foods have been used. This avoids monotony but is open to the objection that fluctuations in the intake of various factors — both quantitative and qualitative — are constantly occurring. Najjar, Holt and their collaborators ('43, '44, '46) avoided this difficulty by employing a purified or "synthetic" diet prepared from vitamin-free casein, vitamin-free dextrimaltose, a hydrogenated vegetable fat, a mineral mixture and a vitamin mixture containing the known essential factors. Monotony did not prove a serious drawback and the use of this diet was found to be consistent with the maintenance of good health for periods of over a year. It was therefore felt by the present authors that it could safely be employed with infants.

[1] This study was aided by grants from the American Dairy Association, the Sugar Research Foundation, and the Dazian Foundation for Medical Research.

[2] Mead Johnson fellow in pediatrics.

Studies on the B vitamin requirements of infants on a similar purified diet were undertaken by us, some of which are reported elsewhere (Holt et al., '49; Snyderman et al., '49). In these studies it was not planned to induce deficiency, since that was not felt to be justifiable. The vitamin requirements were evaluated by urinary excretion data involving adjustment of intake until a "point of minimum excretion" was obtained which presumably represented the minimum adequate intake. In all, some 40 infants have been so fed, most of them for periods no longer than 4 or 5 months. These infants gained weight normally and exhibited every sign of health. In a few instances, however, in which the diet was employed for a longer period — over 6 months — we made observations which led us to question the adequacy of the diet. A number of these infants, although still appearing normally healthy, showed an arrest of weight gain which could be restored by a return to a normal mixed diet. Periods of temporary cessation of growth are commonly observed in normal infants. During the second half year these often last for a week or two and occasionally longer, without apparent cause. We were eventually convinced that we were not dealing with such a normal "arrest," by the duration of the episode and by the prompt and consistent response to natural food or, in some instances, to supplements. Because of the obvious implication of these findings, pointing to the existence of an unknown human dietary essential, it has seemed desirable to report in detail our observations on 11 infants who have exhibited this phenomenon.

## PRODECURES

### *Subjects*

The subjects of this report were infants and young children varying from three and one-half to 40 months of age and weighing from 4.0 to 12.7 kg. Five infants were entirely normal; the remainder, largely the older group, were afflicted with various degrees of mental deficiency. All were

kept on metabolism frames for 5 days a week during the period under discussion, being subjects in which one or another B vitamin requirement was being evaluated by urinary excretion data. As explained above, deficiency of the particular vitamin under study was not allowed to occur.

### *Diet*

The basal purified ration consisted of vitamin-free casein,[3] a partly hydrogenated vegetable fat,[4] a vitamin-free preparation of dextrimaltose [5] and a salt mixture (table 1). These ingredients were mixed together with sufficient water to make a semifluid consistency. The diet was so constructed as to provide 100 cal. per kilogram, with the following percentage distribution of calories: protein 15%, fat 35%, carbohydrate 50%. The diets were identical in all instances except in the case of subject 11, who recived isocaloric quantities of raw egg white in place of vitamin-free casein.

All vitamins were supplied as supplements. Fifteen drops of oleum percomorphum were given daily to provide vitamins A and D. The water soluble vitamins were supplied as a mixture, divided into 4 equal doses given midway among the 5 feedings. Two different mixtures were used. Subjects 1 to 6 inclusive received a mixture (vitamin mixture I, table 1) which was designed on the basis of the knowledge then available of adult requirements. This was subsequently found to be inadequate in riboflavin (Snyderman et al., '49) and ascorbic acid (Holt et al., '49). A second mixture (vitamin mixture II, table 1) was then substituted, designed to supply daily quantities of B factors corresponding to those in one quart of cow's milk. Folic acid was included in this mixture.

### *Examinations*

All infants were carefully observed by the medical and nursing staff, and were given daily physical examinations.

[3] Labco.

[4] Crisco. Supplied by courtesy of Procter and Gamble.

[5] Specially prepared by Mead Johnson and Company.

TABLE 1

*Mineral and vitamin components of diets*

| COMPOSITION OF THE SALT MIXTURE | | COMPOSITION OF VITAMIN MIXTURE I | | COMPOSITION OF VITAMIN MIXTURE II | |
|---|---|---|---|---|---|
| | % | | mg | | mg |
| NaCl | 18.9 | Thiamine | varied | Thiamine | 0.38 |
| $CaH_2PO_4$ (anhydrous) | 25.0 | Riboflavin | 0.167 | Riboflavin | 2.0 |
| $MgSO_4$ (anhydrous) | 6.8 | Nicotinamide | 4.16 | Nicotinamide | 0.85 |
| $KHCO_3$ | 44.4 | Pyridoxine hydrochloride | 0.167 | Pyridoxine hydrochloride | 0.67 |
| KCl | 2.88 | Calcium pantothenate | 0.167 | Calcium pantothenate | 3.5 |
| $FeC_6H_5O_7$ (ferric citrate) | 2.21 | Para-aminobenzoic acid | 0.167 | Para-aminobenzoic acid | 0.5 |
| $CuSO_4$ (anhydrous) | 0.24 | Choline chloride | 0.833 | Choline chloride | 147.0 |
| $MnSO_4$ (anhydrous) | 0.15 | Inositol | 0.167 | Inositol | 180.0 |
| KI | 0.015 | Ascorbic acid | 4.0–12.0 | Ascorbic acid | 25.0 |
| NaF | 0.03 | | | Folic acid | 0.05 |

Routine blood and urine examinations, complete ophthalmological studies, electrocardiograms, roentgenograms of the chest and long bones, and measurements of growth were made at monthly intervals and in some instances more frequently.

### CLINICAL OBSERVATIONS

Of the 11 subjects fed as described, one infant (7) continued to gain weight normally throughout the entire period of study (175 days). Six of the remaining 10 cases followed a fairly definite pattern, consisting of normal weight gain for 100 to 180 days followed by a period of stationary and then of receding weight. Of the remaining 4, three developed weight arrest at about the 40th day and the 4th failed to gain at all but maintained his weight for 220 days. Protocols of individual subjects are given below. Their weight curves are reproduced in figures 1 to 11. None of these infants showed any change in his hematological status. Hemoglobin and red blood cell levels were maintained well throughout the period of study and there were no alterations in the white cell series.

### COMMENT

The failure of these subjects to gain weight bore no relationship to the variations in intake of the vitamins being studied at the time. The possible role of intercurrent infections remains to be considered. No intercurrent infections of any kind occurred in 6 of the 10 cases exhibiting arrest of weight. In two instances (cases 5 and 10) intercurrent illness may well have precipitated the weight arrest, which was not resumed after the infection had subsided. In two other instances (cases 6 and 9) intercurrent infections occurred but seemed to have no relation to the failure to gain weight. In case 6 the infection developed two months after the first failure to gain was noted, and in case 9 weight gain was resumed after an infection which occurred around the 90th day and continued until its final arrest at about the 180th day.

That failure to gain weight or loss of weight in these studies was due to a nutritional defect is indicated by dietary supplementation studies which it was possible to carry out in 8 of

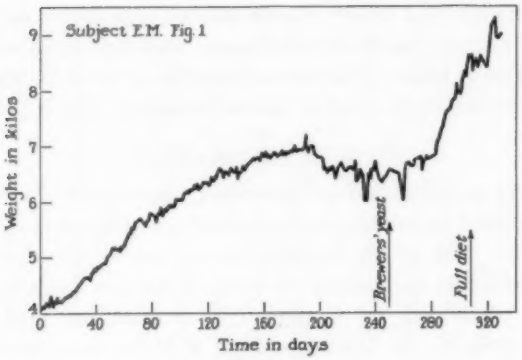


Figure 1

Case 1, E.M. (fig. 1) age 5 months, weight 4.05 kg, normal infant, prematurely born, on study to determine thiamine requirement of the infant, given vitamin mixture I. He had an excellent weight gain for 154 days and maintained this weight until the 200th day, when he started to lose weight slowly. Supplementation of the purified regime with brewers' yeast on the 255th day resulted in a weight gain beginning on the 283rd day which was well sustained. Substitution of a normal diet for the purified diet had no further beneficial effect.

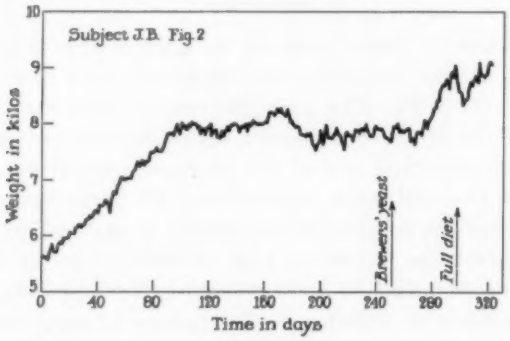


Figure 2

Case 2, J.B. (fig. 2), age 3.5 months, weight 5.65 kg, normal infant, on study to determine thiamine requirement, given vitamin mixture I. He had an excellent weight gain for the first 100 days and maintained his weight until the 180th day, when it started to decline. Brewers' yeast supplementation on the 252nd day was followed by a weight gain in 26 days, which was well sustained. Institution of a normal diet was followed by a slight weight loss because of the infant's refusal to eat unaccustomed food, but the weight resumed its upward swing when the intake became adequate.

the 10 cases showing one or the other phenomenon. In the remaining two this could not be done; in one instance because of an intercurrent infection sufficiently serious to warrant cessation of the study, and in the other because of a social problem necessitating discharge. The supplementation studies have not provided a conclusive answer to the question of the nature of the dietary defect, but they have given some suggestive information.

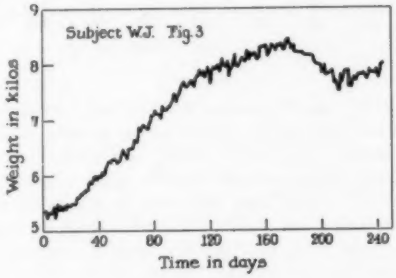


Figure 3

Case 3, W.J. (fig. 3), age 4 months, weight 5.4 kg, normal infant, subject on study to determine thiamine requirement, given vitamin mixture I. He had a good weight gain for 180 days, followed by a gradual weight loss.

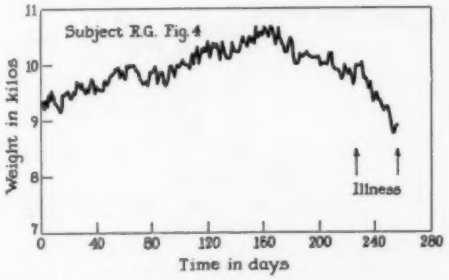


Figure 4

Case 4, R.G. (fig. 4), age 20 months, weight 9.38 kg, mentally defective, on study to determine thiamine requirement, given vitamin mixture I but received 1.5 times the daily dose because of large size. He had a weight gain compatible with his age for 166 days and then a gradual decline. Weight loss during the last 15 days was caused by an unassociated febrile illness.

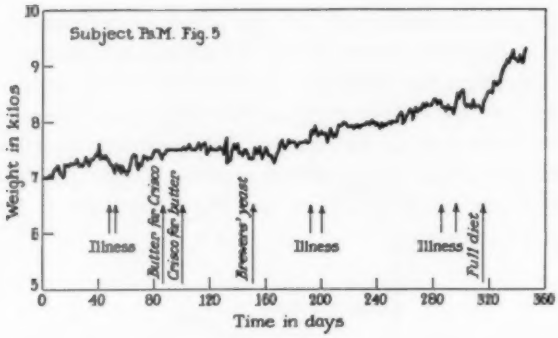


Figure 5

Case 5, P.S.M. (fig. 5), age 6 months, weight 7 kg, normal infant, prematurely born, on thiamine requirement study, given vitamin mixture I. He had a fair weight gain for the first 80 days; one period of weight loss during this initial period was associated with an upper respiratory tract infection. He maintained his weight from the 80th to the 149th day when brewers' yeast supplementation was begun. He had a gain in weight after 22 days which was continued until the 315th day, when a normal diet was substituted. This gave the weight gain an added spurt, which slowed down after 14 days to the same rate of gain as was produced by the brewers' yeast.

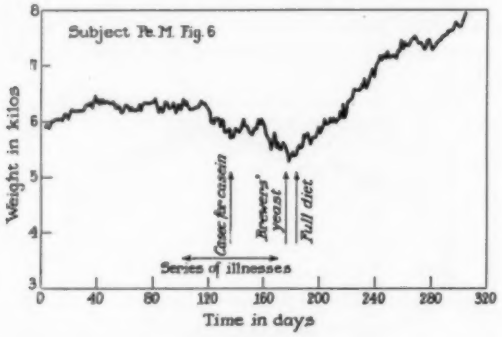


Figure 6

Case 6, P.E.M. (fig. 6), age 6 months, weight 5.75 kg, twin brother of P.S.M., normal infant, prematurely born, on thiamine requirement study, given vitamin mixture I. He gained weight for 40 days; then his weight was stationary until the 120th day, when it started to decline. His course was complicated by repeated upper respiratory tract infections and attacks of otitis media. Substitution of casec for vitamin-free casein had no effect on the weight curve. Brewers' yeast was given for only 6 days, without any effect. A normal infant diet produced an immediate effect on the weight curve.

It is clear from observations of cases 5, 6, 8 and 11 that a normal mixed diet caused an immediate resumption of weight gain, as would be anticipated. The addition of brewers' yeast [6] (15 gm per day) produced a somewhat inconstant weight response after a lag period of 10 to 28 days. In cases 1 and

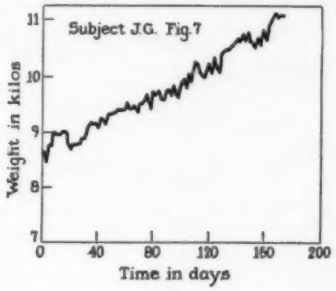


Figure 7

Case 7, J.G. (fig. 7) age 22 months, weight 8.62 kg; diagnosis: arrested hydrocephalus; on study of riboflavin requirement of the infant, given vitamin mixture II. Excellent weight gain for all 174 days he was fed purified diet.

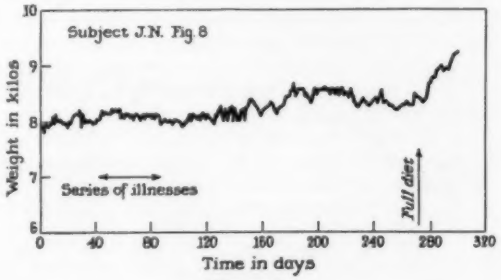


Figure 8

Case 8, J.N. (fig. 8), age 14 months, weight 7.95 kg; diagnosis: kernicterus, mental deficiency; on study to determine riboflavin requirement, given vitamin mixture II. He never had a true weight gain except for one period of slight gain between the 160th and 180th days. His failure to gain weight at the onset was related to frequent attacks of otitis media and bronchitis. He was given a normal infant diet on the 274th day, which produced an immediate effect on the weight curve.

[6] The brewers' yeast was dried yeast purchased from the Brewer Company.

2 this gain in weight was altogether satisfactory. In cases 9 and 5 a definite response occurred, again after a lag period, but the response was a subnormal one. The incompleteness of the response in the last instance may have been due to

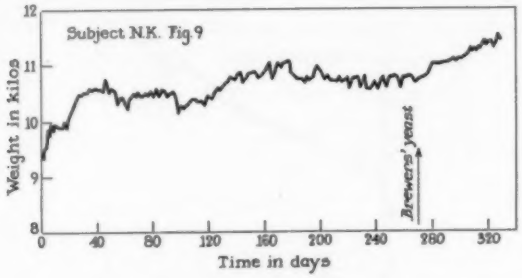


Figure 9

Case 9, N.K. (fig. 9), age 30 months, weight 9.42 kg; diagnosis: mental deficiency; on study to determine riboflavin requirement, given vitamin mixture II. He had a weight gain compatible with his age until the 180th day, when it began to fall off slightly. Brewers' yeast supplementation on the 270th day brought about a beneficial result in 10 days.

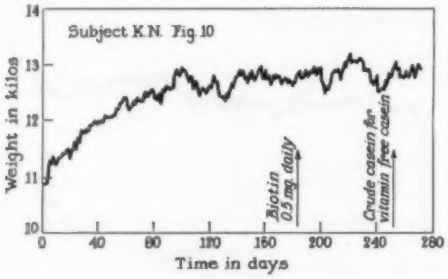


Figure 10

Case 10, K.N. (fig. 10), age 40 months, weight 10.9 kg; diagnosis: mental deficiency; on study of the effect of the type of carbohydrate on the riboflavin excretion, given vitamin mixture II. He had an excellent weight gain for 97 days and then maintained this weight except for some minor fluctuations. The substitution of sucrose, galactose and glucose for the dextrimaltose had no effect; lactose gave a slight transitory weight gain. The addition of biotin, 0.5 mg daily, on the 182nd day produced no change in weight. Crude casein in place of vitamin-free casein did not cause a weight gain.

subsequent intercurrent infection. In case 6 the yeast supplement was continued for only one week, during which time no striking effect occurred.

A few observations were made with other dietary supplements. In case 5 butter was substituted for hydrogenated vegetable fat for 14 days without any improvement in the weight curve, suggesting that no peculiarity of the fat was at

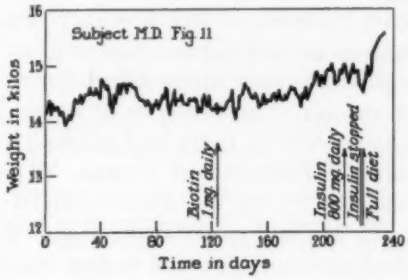


Figure 11

Case 11, M.D. (fig. 11), age 25 months, weight 14.1 kg; diagnosis: mental deficiency; on study of biotin deficiency, given vitamin mixture II. The diet of this baby was similiar to the basal ration except that raw egg white was substituted for the vitamin-free casein. He had a good weight gain for 40 days and thereafter maintained his weight within rather narrow limits. Biotin, 1 mg daily, was added on the 124th day without effect on the weight curve; high urinary biotin values at this time suggested that adequate quantities were being absorbed. The oral administration of 800 mg of insulin per day to provide strepogenin from the 215th to the 227th day was without appreciable effect. The substitution of a normal diet for the purified ration on the 228th day resulted in a prompt increase in weight.

fault. The addition of 0.5 mg of biotin a day to the diet of case 10 failed to influence the weight gain. The possibility of damage to some amino acid or important polypeptid complex in the preparation of the vitamin-free casein [7] was also considered. In order to exclude a possible deficiency of strepogenin, 0.8 gm per day of insulin was given orally to

[7] The vitamin-free casein was assayed for strepogenin through the kindness of Dr. D. W. Woolley and found to contain the same amount of this factor that is present in crude casein.

case 11 for 12 days; no effect was observed. Cases 6 and 10 were given preparations of crude casein which no attempt had been made to render vitamin-free; these were without evident effect.

These results would seem to indicate that the human infant requires for growth some soluble factor which must be present in milk and which is not any of the recognized accessory factors. It appears to be present, to some extent at least, in brewers' yeast. Turning to the literature on animal nutrition for suggestions as to what this factor might be, one finds many reports dealing with unidentified growth factors for one or another species. The presence of a new growth factor in liver which is needed by foxes and minks (Schaefer et al., '48a, '48b), pigs (McRoberts and Hogan, '44; Bustad et al., '48), dogs (Schaefer et al., '42; Frost and Dann, '44), mice (Bosshardt et al., '46) and rats (Geyer et al., '47), in addition to the established accessory factors, is now indicated by many recent studies. Whether this "animal protein factor" in liver is identical with similar factors obtained from crude casein (Cary et al., '46), fish solubles (Zucker and Zucker, '48), distillers' solubles (Novak and Hauge, '48) and cow manure (Rubin and Bird, '47) remains to be proved. The possible identity of this factor with the recently isolated antipernicious anemia factor, vitamin $B_{12}$, is under investigation in a number of laboratories. This last-named vitamin has been shown to be a growth factor for the chick by Ott et al. ('48) and for the rat by Emerson and associates ('49). Studies are needed to throw light upon the question of whether or not the human factor of which evidence is presented above is identical with vitamin $B_{12}$ or the "animal growth factor."

## SUMMARY

A deficiency syndrome which occurs after prolonged feeding of a purified ration in which all the vitamins are supplied as synthetic supplements is described. The syndrome consists of a cessation of weight gain unaccompanied by any other symptoms or signs. A normal infant diet brings about an

immediate weight gain; supplementation with brewers' yeast also has a beneficial effect after a preliminary lag period of 10 to 28 days.

The existence of an unknown factor contained in the normal diet and necessary for human growth has been recognized. This factor may be identical with vitamin $B_{12}$ or the "animal growth factor"; whether or not it is, remains to be seen.

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# FURTHER STUDIES ON THE ROLE OF POTASSIUM IN GROWTH AND BONE FORMATION [1]

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The importance of potassium in the nutrition of the chick was demonstrated by Dor ('41), who showed that a deficiency of this element resulted in retarded growth and high mortality. The symptoms exhibited by potassium-deficient chicks have been described by Gillis ('48), who found that White Leghorn cockerels required 0.20 to 0.24% potassium in the diet to the age of 4 weeks. In the latter work, evidence was obtained that the potassium requirement is related to the phosphorus content of the diet, and that an inadequate intake of potassium may result in reduced bone calcification.

In the present paper the results are reported of further studies on the role of potassium in growth and bone calcification in both chicks and rats. Particular attention has been directed to the interrelation between potassium and phosphorus and the effect of potassium on the metabolism of the bone forming elements. In view of the apparent efficacy of biotin in relieving, at least temporarily, the symptoms of potassium deficiency in dogs (Smith, '44, '45) and calves (Flipse et al., '48), the possible effect of biotin on these symptoms in chicks was also investigated.

[1] Supported in part by a grant from the International Minerals and Chemical Corporation, Chicago, Illinois.

[2] Resident Doctor.

## CHICK EXPERIMENTS

### *Procedure*

White Leghorn male chicks were used in both experiments. They were wing-banded and placed in electrically heated battery brooders when approximately 12 hours old, and allowed free access to feed and water. As is indicated in table 1, the number of chicks started per lot varied somewhat depending on anticipated mortality and differences in rate of growth. Individual weights were taken at weekly intervals during the experimental periods, which were of 4 weeks duration. At the conclusion of the experiments blood samples, pooled for each lot, were analyzed for calcium and phosphorus and the per cent ash in individual bones was determined according to the procedure prescribed by the Association of Official Agricultural Chemists ('45).

The experimental diets had the following percentage composition: water-washed dried blood fibrin, 20; gelatin, 4; ground cellophane, 3; soybean oil, 3; fish liver oil, 0.5; liver "L,"[3] 1; salt mixture, 0.8; vitamin mixture, 0.4; dicalcium phosphate, calcium carbonate and potassium chloride were used in amounts required to give the desired quantities of Ca, P and K, and corn starch was added to adjust all rations to 100%. The basal mixtures were analyzed for potassium by means of flame photometry.

The results of two experiments with chicks are reported. In the first experiment graded quantities of potassium were added to diets containing 0.3% and 0.6% phosphorus. In order to avoid any effects due to variation in calcium intake, the calcium received by all lots amounted to 0.75% of the diet. In the second experiment graded quantities of potassium were added to diets containing 0.35% and 0.6% phosphorus, the calcium-phosphorus ratio being maintained at two.

[3] Wilson's.

## RESULTS AND DISCUSSION

*Growth and livability.* The effects of feeding graded amounts of potassium on the growth and survival of chicks are shown in table 1. In the case of chicks receiving marginal or submarginal allowances of phosphorus (0.3 and 0.35%), growth was improved by increasing the potassium in the diet to approximately 0.24%. The results obtained with the more optimum allowance of 0.6% phosphorus were so nearly comparable for both experiments that the data are combined in table 1. The results with fairly large numbers of chicks indicate that with diets containing this amount of phosphorus the potassium requirement for a maximum rate of growth is about 0.2% of the diet. Since optimum quantities of phosphorus are contained in most practical rations, it seems safe to conclude that the requirement for potassium in such rations is approximately 0.2%. Almost any combination of natural feed ingredients will meet this requirement.

These results are in accord with the previous report by Gillis ('48) concerning quantitative requirements for potassium and the effect on this requirement of varying the phosphorus intake. Since the results obtained in two experiments were quite comparable, while wide variations were empolyed in the total calcium in the diet and in the Ca : P ratio, it does not appear that the level of calcium in the diets influenced the potassium requirement.

Mortality was quite severe in chicks receiving 0.12% dietary potassium and no chicks receiving less than this amount survived the 4-week experimental period. In the second experiment, 0.14% potassium resulted in a mortality of 20 to 25%, indicating that this amount is also inadequate for survival in many instances. Mortality occurred in chicks receiving 0.16% potassium only when the phosphorus content of the diet was critically low, 0.3%. Since this allowance of phosphorus is obviously inadequate for growth, mortality in this instance was very probably influenced by phosphorus deficiency.

TABLE 1

*Growth, livability, and feed efficiency of chicks fed graded amounts of potassium when the phosphorus content of the ration was varied*

| DIETARY POTASSIUM | 0.3% P | | | | 0.35% P | | | | 0.6% P | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. chicks | Weight 4th wk. | Mort. | Gm feed/ gm gain | No. chicks | Weight 4th wk. | Mort. | Gm feed/ gm gain | No. chicks | Weight 4th wk. | Mort. | Gm feed/ gm gain |
| % | | *gm* | % | | | *gm* | % | | | *gm* | % | |
| 0.02 | 10 | ... | 100 | ... | | | | | 10 | ... | 100 | ... |
| 0.08 | 15 | ... | 100 | ... | | | | | 15 | ... | 100 | ... |
| 0.12 | 20 | 96 | 80 | 3.53 | | | | | 20 | 95 | 65 | 4.52 |
| 0.14 | .. | ... | ... | ... | 10 | 153 | 20 | 2.90 | 40 | 157 | 25 | 2.73 |
| 0.16 | 20 | 137 | 35 | 2.73 | .. | ... | .. | ... | 20 | 199 | 0 | 2.10 |
| 0.18 | .. | ... | ... | ... | 10 | 229 | 0 | 2.15 | 30 | 247 | 0 | 1.92 |
| 0.20 | 20 | 182 | 0 | 2.11 | 10 | 237 | 0 | 2.45 | 40 | 282 | 0 | 1.80 |
| 0.22 | .. | ... | ... | ... | 10 | 266 | 0 | 1.91 | 20 | 293 | 0 | 1.78 |
| 0.24 | 15 | 210 | 0 | 1.91 | 10 | 271 | 0 | 1.85 | 15 | 290 | 0 | 1.79 |
| 0.28 | 10 | 205 | 0 | 1.99 | | | | | 10 | 274 | 0 | 1.91 |

*Feed efficiency.* The data in table 1 also show that economy of feed utilization was markedly reduced in the absence of an optimum amount of dietary potassium. It appears that the feed attained its maximum efficiency in producing a unit of gain when it contained approximately the same percentage of potassium as that required for maximum growth under the same conditions. Thus, the efficiency of the basal diet in producing gains when it contained a suboptimum amount of phosphorus was increased by increments of potassium up to about 0.24%, while with an optimum amount of phosphorus the maximum efficiency was realized at around 0.2% potassium.

TABLE 2

*Per cent ash in bones and amounts of calcium and phosphorus in blood plasma from chicks fed graded increments of potassium*

| DIETARY POTASSIUM | 0.35% P | | | 0.60% P | | |
|---|---|---|---|---|---|---|
| | Bone ash | Plasma inorganic P | Plasma Ca | Bone ash | Plasma inorganic P | Plasma Ca |
| % | % | *mg* % | *mg* % | % | *mg* % | *mg* % |
| 0.12 | ... | ... | ... | 36.4 | 4.70 | 11.2 |
| 0.14 | 30.4 | 3.78 | 12.4 | 38.4 | 4.51 | 11.9 |
| 0.16 | ... | ... | ... | 48.4 | 6.80 | 10.9 |
| 0.18 | 37.1 | 4.00 | 12.5 | 47.1 | 6.95 | 12.2 |
| 0.20 | 42.3 | 5.34 | 12.0 | 47.7 | 7.34 | 11.8 |
| 0.22 | 41.7 | 5.25 | 11.9 | 47.6 | 7.40 | 11.9 |

*Bone calcification and blood Ca and P.* Previous observations with a limited number of chicks suggested the importance of potassium in the bone calcification process. The data in table 2 from a large number of chicks fully confirm this observation. The differences between values obtained for bone ash in lots receiving 0.14% potassium or less and lots receiving more of this element were highly significant statistically when examined by the method of "Student" for unpaired data. The difference between the lots receiving 0.18% and 0.20% potassium was also significant when the diet contained only 0.35% phosphorus. The data in this table

also afford striking evidence of the interrelationship between the amount of phosphorus in the diet and the requirement for potassium. Chicks receiving 0.6% phosphorus clearly required not more than 0.16% potassium for maximum calcification, while those receiving 0.35% phosphorus required 0.20% potassium for this function.

The decrease in degree of calcification of the bones of chicks receiving insufficient potassium cannot be explained on the basis of reduced rate of growth or retarded development. The data in table 3 show that chicks making widely different rates of gain may be quite similar with respect to bone ash, and vice versa. The processes of over-all body growth and calcification are, therefore, not directly correlated.

The data in table 2 also lend further support to the hypothesis that the effect of potassium on calcification is mediated through its influence on phosphorus metabolism rather than on calcium metabolism. The more inadequate intakes of potassium caused the amount of inorganic phosphorus in the plasma to be depresed by as much as 30 to 35%. There was no similar depression of the calcium content in these experiments. The depression of plasma inorganic phosphorus occurred in spite of the kidney damage caused by potassium deficiency (Gillis, '48), a condition which is generally associated with elevated blood phosphorus. That potassium ions are concerned in the functioning of certain phosphorylating enzymes has already been indicated by the work of Boyer, Lardy and Phillips ('43) and Nachmansohn and John ('44). On the basis of the present work it appears likely that potassium is also concerned in the retention and deposition of phosphorus in the body tissues.

*Ineffectiveness of biotin in preventing or relieving symptoms of potassium deficiency in chicks.* There has been some debate concerning the ability of either potassium or biotin to relieve certain deficiency symptoms in other species than chicks. Smith ('44, '45) described a progressive ascending paralysis in dogs which responded promptly to synthetic biotin therapy. Using the same diet, Ruegamer et al. ('46)

presented evidence that a deficiency of potassium rather than one of biotin was involved in Smith's work, and that the response to biotin therapy was transitory at best. Flipse et al. ('48) have described a progressive type of paralysis in calves fed a synthetic diet in which cerelose was used as the source of carbohydrate. Prompt responses were obtained in these calves from either the oral administration of a potassium salt or the subcutaneous injection of biotin.

TABLE 3

*Weight and per cent of bone ash of chicks* [1]

| AVERAGE WEIGHT OF GROUPS OF CHICKS AT 4 WEEKS | AVERAGE PER CENT BONE ASH FOR SAME CHICKS |
|---|---|
| *gm* | % |
| 95 | 36.4 |
| 96 | 32.3 |
| 137 | 37.8 |
| 153 | 30.4 |
| 199 | 48.4 |
| 220 | 32.3 |
| 229 | 37.1 |
| 247 | 47.1 |
| 251 | 35.0 |
| 254 | 33.5 |

[1] These data, presented to suggest the lack of correlation between growth and calcification, are selected from published and unpublished material of the author.

In view of these results with dogs and calves, it was decided to study the effect of biotin on the development and relief of symptoms exhibited by chicks on a potassium-low diet. Accordingly, comparisons were made between chicks receiving 0.1 μg of biotin per gram of potassium-deficient diet and chicks receiving 1.1 μg of this vitamin in the same diet. The lower allowance is the minimum of this factor recommended in practice. Chicks in another lot receiving 0.1 μg of biotin per gram of diet were each injected with 1 mg of biotin on the 11th and 16th days.

In table 4 the comparative data on mortality, growth and calcification of chicks receiving these various treatments are

TABLE 4

*The results of administering supplementary biotin to chicks deficient in potassium*

| DIETARY POTASSIUM | BASAL DIET (0.1 μG BIOTIN PER GRAM) | | | | BASAL + 1.0 μG BIOTIN PER GRAM | | | | BASAL + 1 MG BIOTIN INJECTED 11TH AND 16TH DAYS | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. chicks | Mort. | Weight | Bone ash | No. chicks | Mort. | Weight | Bone ash | No. chicks | Mort. | Weight | Bone ash |
| % | | % | *gm* | % | | % | *gm* | % | | % | *gm* | % |
| 0.06 | 15 | 100 | ... | ... | 10 | 100 | ... | ... | | | | |
| 0.14 | 18 | 17 | 151 | 37.1 | 10 | 20 | 157 | 37.9 | 10 | 20 | 165 | 40.0 |

presented. It is evident from the data that supplying biotin greatly in excess of the ordinary requirement, either in the diet or by injection, resulted in no significant benefits to chicks suffering from potassium deficiency. Subcutaneous injections of biotin were given a number of chicks which developed the paralytic symptoms associated with potassium deficiency; such injections, however, were completely ineffective in relieving these symptoms.

## RAT EXPERIMENT

### *Procedure*

In order to extend the study of the relationship between phosphorus and potassium requirements to another species, rats were used in an experimental design similar to that described above for chicks. Weanling male rats of the Sprague-Dawley strain were used. Each experimental group consisted of 9 animals, except that the basal groups receiving no supplemental potassium contained three animals. The experimental diets contained dextrose, 42%; blood fibrin, 25%; gelatin, 5%; commercial hydrogenated cottonseed oil,[4] 8%; liver "L," 1%, vitamin mixture, 0.6% and salt mixture, 0.8%; dicalcium phosphate and potassium chloride were added to secure the various levels of phosphorus and potassium indicated in table 5, and calcium carbonate was added to adjust the Ca:P ratio to 2:1. After the addition of the latter mineral supplements, corn starch was added to bring the mixture up to 100%.

In this experiment graded amounts of potassium were added to the basal diet containing 0.2, 0.3, and 0.4% phosphorus. Previous work [5] had shown that approximately 0.35% of available inorganic phosphorus gives optimum results with this strain of rats. The dietary levels of phosphorus selected for use in this work, therefore, provided a range varying from somewhat deficient to slightly above the minimum re-

[4] Crisco.

[5] Unpublished data of the author.

quired for optimum results. The quantities of potassium used also covered a range which varied from severely deficient to more than the minimum requirement. The rats were maintained on the experimental diets for 4 weeks. At the end of this time the final individual weights were taken, all rats were sacrificed and the left femurs were removed for the purpose of determining the per cent of bone ash. The determinations were made on individual bones by the same procedure used for chick bones.

TABLE 5

*Growth and bone calcification in young rats fed different amounts of potassium in diets of varying phosphorus content*

| DIETARY POTASSIUM | 0.2% P | | 0.3% P | | 0.4% P | |
|---|---|---|---|---|---|---|
| | Wt. gain | Bone ash | Wt. gain | Bone ash | Wt. gain | Bone ash |
| % | *gm* | % | *gm* | % | *gm* | % |
| 0.05 [1] | 36 | 44.7 | 37 | 48.0 | 48 | 53.8 |
| 0.13 | 117 | 48.9 | 116 | 51.9 | 125 | 54.5 |
| 0.16 | 130 | 47.6 | 138 | 54.8 | 147 | 57.8 |
| 0.19 | 140 | 50.9 | 141 | 55.3 | 147 | 57.7 |

[1] Basal lots contained three rats, all other lots 9 rats.

## *Results*

The results obtained with respect to growth and bone calcification by varying the amounts of potassium and phosphorus in the diet are given in table 5. It is obvious that both elements were concerned in growth and bone formation within the dietary ranges studied. While the effect of potassium on bone formation was evident, it was less striking for the rat than for the chick. This may be accounted for in part by the fact that bone formation in young rats old enough for experimental feeding has proceeded considerably further than in the chick at time of hatching. A deficiency of the bone-forming elements is much more serious in the very young chick than in the weanling rat.

It is evident that in this experiment more phosphorus was required for bone formation than for growth. For example, with 0.19% of potassium in the diet there were no significant differences in the weight gains of rats receiving 0.2% phosphorus and those of rats receiving 0.3 or 0.4% phosphorus. There was, however, a significant difference in the per cent of ash found in the bones of rats from these lots. Increasing the phosphorus in the diet produced a progressively greater degree of calcification for any given amount of potassium.

It also appears that the potassium requirement of the rats was affected by the phosphorus content of the diet. Diets containing 0.2% phosphorus gave best results when they also provided 0.19% potassium. On the other hand, diets containing 0.3 or 0.4% phosphorus gave optimum results with 0.16% potassium. Since both chicks and rats, in these experiments, have shown the same response in this regard, it appears likely that the metabolic relationship between potassium and phosphorus is a general one.

The results of this experiment indicate that when the diet is adequate in other nutrients, including phosphorus, the potassium requirement of the young rat is about 0.16% of the diet. This is in good agreement with the results of Kornberg and Endicott ('46), who reported a minimum requirement of 0.17% potassium for the rat.

## SUMMARY

1. In these experiments chicks required 0.20% potassium in an otherwise adequate diet for a maximum rate of growth during the first 4 weeks of life. It appears, however, that due to an interrelationship in the metabolism of potassium and phosphorus, the requirement for potassium was increased to as much as 0.24% when the phosphorus content of the diet was suboptimum.

2. Bone calcification, as measured by the per cent of bone ash, was significantly influenced by the amount of potassium in the diet. It appears that diets containing inadequate potassium resulted in reduced calcification due to a disturbance

in phosphorus metabolism. Under such conditions the inorganic phosphorus in blood plasma was significantly reduced, while there was no measurable effect on calcium in the plasma. The amount of potassium required for optimum calcification was less in the presence of an adequate intake of phosphorus than when the phosphorus allowance was suboptimum.

3. Potassium was also concerned in the economy of feed utilization, the maximum feed efficiency being obtained with amounts of potassium which were approximately optimum for growth.

4. Contrary to results reported with certain other species, potassium deficiency was easily produced in chicks receiving adequate amounts of biotin. Supplementary biotin in amounts 10 times the ordinary requirement, administered orally or by injection, was without effect on the development or remission of symptoms of potassium deficiency in chicks.

5. Results obtained with rats confirmed the effect of phosphorus on the potassium requirement for growth and bone calcification as observed in chicks. The potassium requirement of the rats in these studies was approximately 0.16% of the diet. The amount of phosphorus required for maximum bone calcification in the rat was greatly in excess of the amount required for normal growth.

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# EFFECT OF THE DIETARY LEVEL OF TOCOPHEROLS ON THEIR METABOLISM IN SWINE

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The pioneer studies of Evans and Burr ('27), in which the rat bioassay test was applied to tissues from rats, cattle, pigs, and sheep, indicated that vitamin E is widely distributed in the animal body but is never present in very great concentration in any one organ or tissue. Musculature, body fat and liver, in the order named, contained appreciably more vitamin than other tissues. Mason ('42) confirmed the conclusions of Evans and Burr relative to the wide distribution of vitamin E in the body of the rat but indicated that musculature and body fat, which the latter workers considered to possess the greatest concentration of the vitamin, actually contained an amount similar to or less than that present in other tissues and organs, whether the previous intake was low or high.

A few investigators have tried to increase the tocopherol content of animal tissues by increasing the level of tocopherols ingested. Lundberg et al. ('44) and Barnes et al. ('43) demonstrated clearly the ability of the rat to store in the abdominal fats excess tocopherols from a diet which had

[1] The data in this paper are taken from the dissertation submitted by this author for the degree of Doctor of Philosophy, Cornell University, 1950. Present address: Department of Animal Nutrition, The Pennsylvania State College, State College.

been supplemented with alpha-tocopherol. To improve the keeping qualities of meat to be used for human consumption, others have tried by the same means to increase the tocopherol content of the meat. In most of these investigations the resistance of the fat to rancidification was used as a measure of its tocopherol content.

Watts, Cunha and Major ('46), working with swine, found that tocopherol supplementation of a purified ration increased the stability of hog fats only slightly, while no increase was obtained in the case of a ration composed of naturally occurring feedstuffs. In contrast with these latter findings, Carpenter and Lundberg ('49) recently reported that the induction period, as measured by an oxygen absorption method at 100°C., of the fats from pigs that did not receive supplemental tocopherols during the post-weaning period was much shorter than the induction period of fats from pigs fed supplementary tocopherols. Their animals received larger supplements of tocopherols over a longer period of time than did those of the former investigators. Major and Watts ('47) found that when high levels of tocopherols were fed to or injected in rabbits on a purified diet, protection from the development of rancidity in the meat and fat was shown. On a natural ration, no protection from the development of rancidity was shown when the animals were injected with tocopherols or tocopherol phosphate three weeks before slaughter. Criddle and Morgan ('47) used both chemical and bioassay methods to show that the tocopherol content of turkey tissues increased significantly as a result of tocopherol feeding. They found that the tocopherol deposits, which were widely distributed throughout the tissues but most concentrated in the liver and heart, represented only a small fraction of the vitamin which had been fed.

The investigation presently reported was designed to determine to what extent the tocopherol content of the organs and tissues of swine could be increased by supplementing a vitamin E-low, purified ration with a concentrate of mixed tocopherols. By using molecular distillation as a step in the

procedure for the chemical estimation of the tocopherols, it appeared possible that on the basis of the distribution of the tocopherols throughout the organs and tissues some insight might be gained as to their role in metabolism.

## EXPERIMENTAL

Five weanling barrows were used as experimental animals. They weighed from 32 to 42 lb. and came from three different litters. Animals were designated as numbers 5, 6, 7, 8 and 9, with the first two serving as controls and the last three receiving a supplement of a concentrate of mixed tocopherols. The supplement was fed to the three animals to supply mixed tocopherols as follows: no. 7 — 2.87 mg, no. 8 — 55.12 mg and no. 9 — 110.2 mg per kilogram live weight daily. Animals were weighed weekly and the amount of supplement was calculated each week.

The animals were kept in individual pens and were hand-fed. The supplement was weighed daily and mixed with the ration that was fed in the evening. The purified ration consisted of 250 parts of casein, 250 parts of corn sugar, 403 parts of starch, 40 parts of roughage,[2] 50 parts of mineral mixture, 5 parts of a liver fraction,[3] and two parts of methionine. The ration was supplemented with vitamins, in milligrams per kilogram of ration, as follows: thiamine hydrochloride, 2.1; riboflavin, 3.1; niacin, 10.3; calcium pantothenate, 14.8; pyridoxine hydrochloride, 2.4; folic acid supplement, 0.6; choline chloride, 1.0; and carotene, 1.2.

The animals were slaughtered after 75 days on the experimental diet. A quart of blood was obtained from each animal, of which a small part was used for determination of vitamins A and E in the plasma and the remainder was lyophilized and ground in a Waring Blendor for tocopherol analysis. The liver, heart, kidneys, and spleen were obtained as soon as the carcasses had been inspected by a veterinarian. Samples of ruffle fat were obtained at the time of slaughter

[2] Ruffex.

[3] Wilson's liver L fraction.

and samples of the leaf, back, jowl, and ham facing fats and a rib chop from each animal were obtained when the carcasses were cut up for processing.

The organs and rib chops were lyophilized and the former were ground in a Wiley mill while the lean portions of the latter were ground in a Waring Blendor with dry ice to prepare them for subsequent analyses. The samples of the different fats were ground in a Waring Blendor with dry ice and then rendered in a water bath at a temperature well below 100°C. The resulting mixture of connective tissue and fat was centrifuged and the supernatant layer of fat was removed by siphoning it off into a small Erlenmeyer flask. All samples were stored at 0°F. while awaiting analysis.

## ANALYTICAL PROCEDURES

The 4-unit molecular semi-micro still as designed and described by Quaife and Harris ('46) was used for the distillation of the tocopherols from the fat or lipid extract. The lipid extracts of the organs, muscle, and whole blood were obtained by using an extraction apparatus similar in design to that described by Quaife and Harris ('48). Samples of 10 gm or less were weighed into Soxhlet thimbles and extracted with 50 ml of hot absolute ethanol for 24 hours. Total tocopherols were measured by the procedure of Emmerie and Engel ('38) on an aliquot of the distillate which had been treated according to the method of Parker and MacFarlane ('40). The diazo method of Quaife ('44) as modified by Weisler et al. ('47) was used to measure gamma-plus delta-tocopherols on another aliquot of the above-treated distillate.

The total tocopherol content of the samples of blood plasma was determined by the method of Quaife and Harris ('44), using the microhydrogenation apparatus of Quaife and Biehler ('45). Blood plasma samples were analyzed for vitamin A by the method of Kimble ('39). Samples of fresh liver were analyzed for vitamin A content by the method of Davies ('33).

## RESULTS

### *Tocopherol content of organs and tissues*

Supplementation with three levels of tocopherols of a purified ration fed to swine, from weanling age until slaughter, increased significantly the content of total tocopherols of all organs and tissues that were studied (table 1). The increases were most marked in the case of the liver and body fats. The values for the total tocopherol content of the organs and tissues from the control animals ranged from zero

TABLE 1

*Tocopherol content*[1] *of organs and tissues*

| TISSUE | HOG NO. | | | | |
|---|---|---|---|---|---|
| | 5 | 6 | 7 | 8 | 9 |
| | Tota. tocopherols μg/gm | | | | |
| Liver | 1.6 | 4.1 | 12.0 | 423.5 | 208.1 |
| Spleen | 7.9 | 1.4 | 10.6 | 53.4 | 64.6 |
| Kidneys | 3.0 | 2.1 | 6.5 | 27.9 | 46.8 |
| Heart | 2.1 | 0.0 | 19.2 | 56.5 | 69.5 |
| Muscle | 2.8 | 4.6 | 18.2 | 41.6 | 124.9 |
| Leaf fat | 21.2 | 4.5 | 66.8 | 214.6 | 284.1 |
| Ruffle fat | 20.9 | 6.2 | 88.1 | 353.3 | 421.7 |
| Jowl fat | 4.5 | 6.2 | 32.7 | 134.6 | 162.6 |
| Back fat | 12.3 | 4.7 | 35.8 | 177.3 | 231.0 |
| Ham facing fat | 0.0 | 0.0 | 32.0 | 176.5 | 198.9 |

[1] Fresh weight basis.

values for heart and ham facing fat up to 21.2 μg/gm for leaf fat. In the case of the supplemented animals, and with only one exception, each increase in the level of tocopherols ingested brought about significant increases in the tocopherol content of all organs and tissues. The liver value for animal 8 of 423.5 μg/gm was the highest value obtained, and was twice the corresponding value for animal 9.

Increases were not noted in the case of the gamma- plus delta-tocopherol content of the organs, tissue, and fats. In no instance was the gamma- plus delta-tocopherol value greater than 5% of the value for total tocopherols. Thus it

is apparent that in our animals the tocopherols were stored chiefly in the alpha form. Similar findings for the hen, cow, and man have been reported by Quaife et al. ('49). On the basis of the above results, the conclusion appears to be justified that tocopherols, in the alpha form, are stored to an appreciable extent by swine when a high level of ingested tocopherols is maintained throughout most of the growing and all of the fattening periods.

TABLE 2

*Tocopherol content of blood plasma and of whole blood*

| | HOG NO. | | | | |
|---|---|---|---|---|---|
| | 5 | 6 | 7 | 8 | 9 |
| Mixed tocopherols (mg/kg live weight daily) | 0 | 0 | 2.87 | 55.12 | 110.2 |
| Plasma (μg/100 ml) | 0 | 0 | 248 | 668 | 594 |
| Whole blood:[1] | | | | | |
| Total (μg/100 ml) | 84 | 90 | 195 | 729 | 647 |
| Gamma plus delta (μg/100 ml) | 0 | 0 | 19 | 34 | 31 |
| Distribution of total tocopherols in:[2] | | | | | |
| Cells (%) | 100.0 | 100.0 | 17.4 | 40.5 | 40.3 |
| Plasma (%) | 0.0 | 0.0 | 82.6 | 59.5 | 59.8 |

[1] Calculated from values obtained on lyophilized whole blood on the basis of a loss on drying of 79.06% moisture.

[2] A hematocrit value of 35% cells was used to calculate the total tocopherol content of the cells and plasma in 100 ml of whole blood.

### *The tocopherol content of blood plasma and of whole blood*

The plasma tocopherol values and the total as well as the gamma- plus delta-tocopherol contents of whole blood are shown in table 2. Plasma tocopherol values and total tocopherol values for the whole blood reflected the level of

tocopherols ingested, with the exception of the highest level. The zero plasma value for the control animals is of considerable interest inasmuch as similar findings have not been reported in the literature for swine. Engel ('49) reported zero plasma values for female rats, which showed resorption sterility in all cases. While sufficient time may not have elapsed for the control animals in our experiment to manifest the symptoms of vitamin E deficiency common to other species of animals, the implications of the above findings are that swine have an extremely low requirement for vitamin E or that plasma tocopherol values do not show accurately the nutritional state of animals with respect to this vitamin, or both.

Tocopherol supplementation increased slightly but significantly the gamma- plus delta-tocopherol content of the whole blood. A zero value was again obtained for the control animals and less than 10% was present in the gamma and delta forms in the case of the supplemented animals. These results further demonstrate the ability of swine preferentially to absorb tocopherol in the alpha form for subsequent storage, and help to explain the greater storage of this form of tocopherol in the organs and tissues which were studied.

A hematocrit value of 35% cells and a moisture content of whole blood of 79.06% were used to make the calculations necessary to show the distribution of tocopherols between the cells and plasma of whole blood. While the control animals had a zero plasma value, the total tocopherol contents of the whole blood, 84 and 90 μg/100 ml, showed that a rather significant quantity was still present in the cells. For this reason it would appear that the chemical estimation of the total tocopherol content of whole blood rather than of plasma might serve as a better basis for evaluating the nutritional state of animals with respect to vitamin E.

### *Fatty acid composition of body fats*

The fatty acid composition of the individual samples of body fats was calculated on the basis of their iodine and

thiocyanogen values, assuming that linolenic and arachidonic acids were absent. The average iodine and thiocyanogen values and the percentages of saturated, oleic, and linoleic acids are presented in table 3.

Tocopherol supplementation of a purified ration, containing at most only traces of fat, brought about significant increases in the iodine and thiocyanogen values of the body fats. These increases manifested themselves in the fatty acid composition of the body fats by raising significantly the percentage of oleic acid at the expense of the saturated fatty

TABLE 3

*Average iodine and thiocyanogen values and fatty acid composition of body fats*

| | HOG NO. | | | | |
|---|---|---|---|---|---|
| | 5 | 6 | 7 | 8 | 9 |
| Iodine value (gm $I_2$/100 gm fat) | 57.1 | 55.0 | 64.0 | 65.8 | 61.0 |
| Thiocyanogen value (gm $I_2$/100 gm fat) | 55.9 | 54.4 | 62.6 | 64.4 | 59.8 |
| Linoleic acid (%) | 1.1 | 0.4 | 1.3 | 1.3 | 1.1 |
| Oleic acid (%) | 64.2 | 63.2 | 71.8 | 73.9 | 68.7 |
| Saturated fatty acids (%) | 34.7 | 36.4 | 26.9 | 24.8 | 30.2 |

acids. The average oleic acid content for the 5 fats from the control animals was 63.7% as contrasted with average values of 71.8, 73.9, and 68.7% for animals 7, 8, and 9, respectively. While the concentrate of mixed tocopherols used as the supplement was composed of one-third of mixed tocopherols and the remainder of an oil rather high in its degree of unsaturation, iodine no. 124.7, the very small amounts of concentrate needed to provide the lowest level of tocopherols ingested could scarcely have accounted for the increase in the percentage of oleic acid at the expense of the saturated fatty acids. Then, too, the two highest levels of tocopherols ingested did not bring about a further significant increase in the degree of unsaturation of the fats; in

fact, there was a slight decrease in the case of the highest level. Ellis ('33) has found that in the synthesis of fats from carbohydrates the predominating fatty acids formed are oleic, palmitic, and stearic in the order named. It would appear that tocopherols may be concerned in the synthesis of oleic acid, inasmuch as the addition of a very small quantity of tocopherols to a vitamin E-low, purified ration resulted in a significant increase in the percentage of this acid which was synthesized.

The occurrence of linoleic acid in the body fats can best be explained on the basis that the amounts found probably represent a conservation of the essential fatty acid present at the time when the animals were placed on experiment. The linoleic acid values show that there was no synthesis of it, which would indicate that swine and rats are alike in their inability to synthesize fatty acids containing more than one double bond (Burr and Burr, '29). Fat from hogs fed a ration low in fat, brewers' rice and a protein supplement had an average linoleic acid content of 1.2% according to Ellis and Isbell ('26). Their analyses of the fat composition of the feed indicated that all of the linoleic acid could be accounted for on the basis of the fat ingested.

## SUMMARY

Five barrows, placed on experiment immediately after weaning, were maintained on a vitamin E-low, purified ration for 75 days. Two animals served as controls and three received a supplement of a concentrate of mixed tocopherols at levels of 2.87, 55.12 and 110.2 mg daily per kilogram live weight. When slaughtered, organs, tissues, body fats, and blood were obtained and analyzed for total and gamma- plus delta-tocopherol contents, using the intermediate step of molecular distillation. Tocopherol supplementation increased significantly the total tocopherol content in all cases studied, while it increased the gamma- plus delta-tocopherol content significantly only in the case of whole blood. Zero values were obtained for blood plasma from the control animals, as con-

trasted with values of 84 and 90 μg/100 ml for whole blood. Tocopherol supplementation markedly affected the fatty acid composition of the body fats by increasing the percentage of oleic acid at the expense of the saturated fatty acids.

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# THE EXCRETION OF AMINO ACIDS IN NORMAL AND UNDERNOURISHED HUMAN SUBJECTS FOLLOWING A SINGLE INFUSION OF AN AMINO ACID PREPARATION [1]

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ONE FIGURE

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The "saturation" or "load" test has been proposed as a means of evaluating the state of nutrition of human subjects with regard to a specific essential nutrient. Under the conditions of this test, subjects possessing a deficient quantity of either thiamine, riboflavin (Melnick, Hochberg and Oser, '45) or ascorbic acid (Belser, Hauck and Storvick, '39) in their tissues were found to excrete smaller amounts of a given dose of a specific vitamin than those with adequate amounts. In both animals (Silber, Seeler and Howe, '46; Sauberlich and Baumann, '46; Pearce, Sauberlich and Baumann, '47) and man (Dunn, Camien, Shankman and Block, '47; Woodson, Hier, Solomon and Bergeim, '48; Eckhardt and Davidson, '49; and Harper, '49), the urinary excretion of individual amino acids has been studied when the subjects were maintained on diets containing varying amounts of protein. In addition, the excretion of individual amino acids has been studied in human subjects in various pathologic states (Eck-

[1] Supported in part by a grant from Merck and Company. A preliminary report of this work was presented at the meeting of the American Institute of Nutrition, Atlantic City, April 17–21, 1950.

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hardt, Cooper, Faloon and Davidson, '48; Frankl, Martin and Dunn, '47). Silber, Seeler and Howe ('46) have reported the urinary excretion of amino acids by normal and protein-depleted dogs which received amino acids intravenously. As far as could be learned, there are no studies on man in which the amino acid excretion has been measured in both normal and undernourished subjects following the infusion of an amino acid preparation. This investigation was undertaken to determine whether the nutritional state of human subjects could be correlated with the excretion pattern of individual amino acids following the intravenous administration of a known amino acid mixture as a test dose.

## METHODS

Four normal and 4 undernourished human subjects were used in this study. One undernourished subject was studied twice. The undernourished patients were chosen on the basis of the following laboratory and clinical criteria: a definite weight loss over a period of up to 6 months prior to the test, a history of inadequate dietary intake, and one or more signs of vitamin deficiency (e.g., red tongue, poor night vision, cheilosis, or peripheral neuritis) which would denote, in general, poor food intake. These subjects were shown to have depleted protein stores by a test developed in this laboratory (Harroun, Smyth and Levey, '50). The normal subjects had none of the above signs or symptoms and were in good health. They had no evidences of recent illnesses and possessed adequate protein stores in terms of the above test.

Each of the subjects underwent the following routine: on the first day of the study, following an 18-hour fast, 1 l of physiological saline was given intravenously at the rate of 20 ml per minute. The saline infusion was used to evaluate the urinary loss of amino acids due to giving fluid intravenously. The urine was collected for a period of 4 hours after the initiation of the infusion. On the second day of the study, the fasting subject received intravenously 500 ml of an amino

acid mixture [3] during a period of 30 minutes. The urine was again collected for a 4-hour period starting with the beginning of the infusion. The amino acid content of both urine samples was estimated.

The following amino acids were determined microbiologically in the amino acid preparation administered and also in the urine samples collected: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine. Tryptophan was not determined in this study. For the estimation of leucine, isoleucine and valine, the method of Hier, Graham, Freides and Klein ('45) was employed. The method of Stokes, Gunness, Dwyer and Caswell ('45) was used for the arginine and threonine estimations. Histidine, methionine, lysine and phenylalanine were determined using the medium of Miller [4] and employing *Leuconostoc mesenteroides*. The free amino acids were considered to be those which were microbiologically available before acid hydrolysis. The combined amino acids were considered to be the difference between the amount of amino acids estimated after and before the acid hydrolysis. The procedure of Woodson, Hier, Solomon and Bergeim ('48) was used to free the combined amino acids.

Two series of controls were used for each assay: one, a negative control, was free of the amino acid to be determined, and the second, a positive control, consisted of a known amino acid mixture diluted to fall within the analytical range used. Recoveries of the individual amino acid studies were within 10%.

## RESULTS

The amount of each of the essential amino acids in the mixture injected is presented in table 1. The values represent the L-form of the amino acids, since the microbiological meth-

[3] The amino acid preparation used in this study consisted of a mixture of amino acids prepared by the recombination and *fortification* of fractions of a casein digest. This material was supplied as a 10% solution in 500-ml units through the courtesy of Dr. Augustus Gibson of Merck and Company, Rahway, New Jersey.

[4] S. Miller, personal communication.

ods employed determine only the natural isomers. The above named amino acids account for approximately 50% of the weight of the amino acids in the mixture; the remainder consists mainly of glycine plus about 0.43% tryptophan and a small amount of non-essential amino acids.

The urinary wastage of the free form of the 9 amino acids assayed, following the infusion of a solution of physiologic saline, is given in table 2. With the possible exception of histidine, there is little difference in the urinary wastage of

TABLE 1

*Amino acid content of the material administered expressed as the L isomer*

| AMINO ACID | MG/500 ML OF SOLUTION |
|---|---|
| Arginine | 2,277 |
| Histidine | 1,004 |
| Isoleucine | 2,796 |
| Leucine | 6,702 |
| Lysine | 5,393 |
| Methionine | 2,550 |
| Phenylalanine | 835 |
| Threonine | 1,111 |
| Valine | 2,912 |

amino acids in normal and undernourished subjects following the saline infusion. The possibility exists that subjects with normal nutrition may excrete more histidine than the undernourished subjects, though there is wide variation in the experimentally determined values in both groups. In general, the amount of amino acid lost in this manner is very small and in some cases approaches the limit of accuracy of the microbiological methods. Usually there was little or no free arginine, methionine, or valine lost in either group following the saline injection.

TABLE 2

*The excretion of amino acids following the infusion of either 1 l of physiological saline or 500 ml of an amino acid mixture*
*The values are expressed as milligrams per 4-hour urine sample*

| PATIENT NUMBER | | ARGININE | | HISTIDINE | | ISOLEUCINE | | LEUCINE | | LYSINE | | METHIONINE | | PHENYLALANINE | | THREONINE | | VALINE | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | F[1] | B[2] | F | B | F | B | F | B | F | B | F | B | F | B | F | B | F | B |
| **Normal subjects** | | | | | | | | | | | | | | | | | | | |
| 1 | S[3] | 0.0 | 0.0 | 30.2 | 0.0 | 0.0 | 14.4 | 0.0 | 5.8 | 5.2 | 2.0 | 0.0 | 0.0 | 1.6 | 2.0 | 0.0 | 7.8 | 0.0 | 0.0 |
| | A[4] | 4.8 | 12.0 | 161.0 | .. | 45.4 | 11.4 | 132.0 | 20.0 | 11.4 | 278.1 | 18.0 | 33.1 | 2.6 | 33.0 | 155.3 | 5.7 | 76.8 | 31.2 |
| 2 | S | 0.0 | 6.7 | 50.0 | 1.2 | 6.4 | 7.0 | 10.7 | 7.8 | 8.2 | 1.4 | 0.0 | 0.0 | 0.8 | 1.6 | 6.0 | 5.0 | 0.0 | 2.9 |
| | A | 17.6 | 21.4 | 246.0 | 8.0 | 167.0 | 0.0 | 132.0 | 218.0 | 263.0 | 262.0 | 197.0 | 0.0 | 83.0 | 0.0 | 335.0 | 0.0 | 283.0 | 0.0 |
| 3 | S | 0.0 | 2.4 | 10.5 | 9.9 | 11.8 | 0.0 | 1.0 | 2.7 | 6.1 | 3.0 | 0.1 | 0.1 | 1.4 | 0.8 | 0 0 | 6.6 | 0.0 | 0.0 |
| | A | 55.6 | 0.0 | 152.0 | 0.0 | 121.9 | 7.0 | 310.0 | 20.5 | 550.0 | 0.0 | 200.0 | 0.0 | 88.2 | 0.0 | 188.0 | 0.0 | 224.5 | 17.0 |
| 4 | A | 0.0 | 14.0 | 1.3 | 150.7 | 54.7 | 12.2 | 181.5 | 28.5 | 188.7 | 239.3 | 65.3 | 2.5 | 29.0 | 6.0 | 172.0 | 18.4 | 149.0 | 31.0 |
| **Undernourished subjects** | | | | | | | | | | | | | | | | | | | |
| 5 | S | 0.0 | 3.2 | 3.2 | 0.8 | 14.3 | 3.1 | 0 0 | 4.5 | 2.9 | 1.4 | 0.2 | 1.8 | 0.0 | 0.0 | 0.0 | 14.1 | 36.4 | .. |
| | A | 26.9 | 9.0 | 165.6 | .. | 75.8 | 7.8 | 149.5 | 5.5 | 500.0 | 15.0 | 110.0 | .. | 45.5 | .. | 165.0 | 12.0 | 0.0 | 157.5 |
| 6 | S | 0.0 | 3.2 | 42.4 | 16.0 | 6.5 | 0.4 | 3.3 | 8.0 | 16.0 | 8.8 | 0.0 | 0.0 | 4.0 | 2.0 | 7.9 | 0.5 | 0.0 | 1.7 |
| | A | 40.0 | 4.2 | 85.5 | 107.5 | 121.0 | 7.0 | 354.0 | | 480.0 | 15.0 | 119.0 | 17.0 | 75.5 | 5.6 | 172.0 | 31.0 | 158.0 | 86.0 |
| 7 | S | 0.9 | 3.0 | 9.8 | .. | 10.8 | 2.5 | 1.5 | 1.7 | 4.3 | | 0.4 | 0.0 | 21.0 | 0.1 | 6.6 | 1.3 | 0.0 | 0.0 |
| | A | 3.0 | 28.5 | 204.0 | .. | 43.5 | 17.5 | 253.5 | .. | 466.0 | 15.0 | 179.0 | 0.0 | 73.2 | 0.0 | 292.0 | .. | 172.5 | .. |
| 7 | S | 0.0 | 5.1 | 60.5 | 68.5 | 6.6 | 2.4 | 3.0 | 9.0 | 5.8 | 8.9 | 0.3 | 0.2 | 3.0 | 1.8 | 3.8 | 9.2 | 0.0 | 3.0 |
| | A | 7.5 | 1.5 | 156.0 | 6.0 | 49.7 | 6.0 | 186.0 | 26.0 | 232.0 | 19.0 | 71.7 | .. | 52.9 | 1.3 | 148.0 | 16.0 | 52.0 | 66.0 |
| 8 | S | 1.2 | 0.0 | 15.1 | .. | 1.2 | 0.0 | 1.2 | 0.0 | 0.4 | .. | 0.2 | .. | 1.1 | .. | 3.5 | 0.0 | 0.7 | 0.0 |
| | A | 1.2 | 1.2 | 115.1 | .. | 17.5 | | 79.3 | 1.2 | 194.6 | .. | 41.5 | .. | 21.0 | | 102.8 | 6.1 | 62.5 | 38.6 |

[1] Free amino acids.
[2] Bound amino acids.
[3] After physiological saline infusion.
[4] Amino acid infusion.

The excretion of free amino acids by the normal and undernourished individuals after the injection of 500 ml of the amino acid mixture is presented in table 2. Despite wide scattering of individual values for the urinary amino acid loss, there appears to be no characteristic difference in loss of an individual amino acid following the intravenous administration of the amino acid mixtures which would aid in

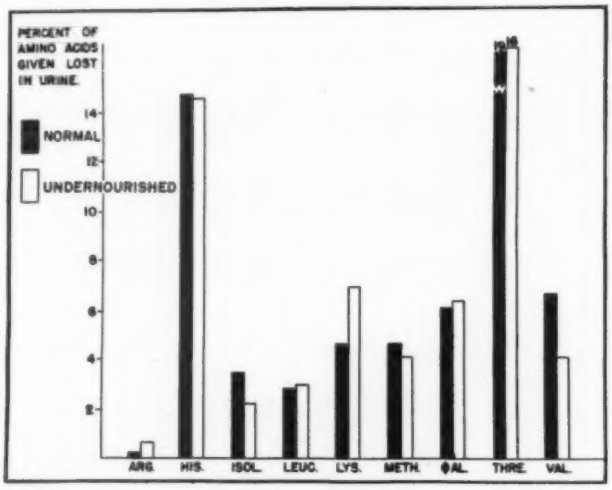


Fig. 1 Average urinary excretion of 9 amino acids after their intravenous administration as an amino acid mixture to 4 normal and 4 undernourished male subjects.

differentiating normal from undernourished states. The percentage of the 9 amino acids infused which was lost by renal excretion during the 4-hour period following the intravenous amino acid injection is given in figure 1. There appears to be some difference in the percentages of lysine and valine lost in the normal and undernourished subjects, but in view of the wide individual variation and the method of determination it is doubtful if these differences are significant. The values for the urinary excretion of the amino acids given in figure

1 agree with the data of Eckhardt and Davidson ('48) except for phenylalanine. The mixture infused in the present study contained less phenylalanine than that used by Eckhardt and Davidson, hence less of the amino acid was excreted.

Since some of the amino acids are excreted in a bound form which may not be microbiologically available, it was of interest to determine the excretion of both types of amino acids. The material infused into the subjects was free of peptides.

TABLE 3

*Retention of administered amino acids; per cent of amount injected*

| AMINO ACID | NORMAL [1] | UNDERNOURISHED [2] |
|---|---|---|
| Arginine | 99.1 | 99.2 |
| Histidine | 86.9 | 85.7 |
| Isoleucine | 96.5 | 97.8 |
| Leucine | 97.1 | 96.9 |
| Lysine | 95.4 | 93.1 |
| Methionine | 95.4 | 95.9 |
| Phenylalanine | 93.9 | 93.6 |
| Threonine | 81.1 | 83.9 |
| Valine | 93.3 | 95.9 |

[1] Four subjects.

[2] Five studies on 4 subjects.

In normal subjects more arginine was usually lost in the bound form than as the free amino acid following the infusion of the amino acid mixture (table 2). After an amino acid infusion increasing amounts of bound lysine and leucine appeared in the urine, particularly in the case of the normal subjects but also to a lesser degree in the undernourished patients. Bound valine was lost by both the normal and undernourished subjects. In all cases there was considerable variation in the amounts of bound amino acids lost from subject to subject.

If one considers the difference between the amounts of any one amino acid given and excreted as the amount retained for metabolic purposes, then the retention of the various amino acids was similar in both groups of patients. The average percentage of the administered amino acids retained varied from 81% for threonine to 99% for arginine, with little difference which could be related to the state of nutrition of the subjects (table 3).

## DISCUSSION

It has been noted that neither the urinary loss of an individual amino acid nor the pattern of excretion of the 9 amino acids assayed could be definitely related to the nutritional status of the individual, since the urinary wastage was similar for the normal individuals and for those who had degrees of undernutrition ranging from marked to mild. Thus, unlike the situation with respect to some of the vitamins, a "load test" (as used in this study) appears to be of little value in estimating the state of protein nutrition.

The data presented in figure 1 show that large percentages of histidine and threonine are lost into the urine, while only traces of arginine are wasted. Similar data were obtained by Eckhardt and Davidson with man ('48, '49) and Silber et al. ('46, '48) with dogs. The latter workers found it was possible to adjust the content of the individual amino acids infused to such a degree that the difference in proportion of the amino acid infused and excreted was eliminated. If a large amount of a given amino acid was excreted it was believed that more than an optimum amount was being injected; on the other hand, if very little of an acid was being lost, it was believed that too little was given; thus it was possible to develop an amino acid mixture which, when infused into dogs, would yield an excretion pattern of amino acids of relatively the same design as the injection pattern. This method could not be used for threonine, since large amounts were excreted into the urine regardless of the proportion infused. The reason for the large urinary wastage of some amino acids following their intravenous infusion is difficult

to explain, since it has been shown by Wright ('48), using dogs, that none of the essential amino acids may be administered at a sufficiently rapid rate to exceed the capacity of the kidney tubules to reabsorb them completely.

There appears to be normally a definite urinary loss of amino acids in the bound form. This occurs to a small extent following a saline injection and increases after the infusion of amino acids. Inasmuch as the mixture infused in this study was free of peptides, the increased amount of bound amino acids must be due to newly synthesized peptides, which have a lower rate of reabsorption in the tubules and thus escape into the urine.

In discussing the excretion of individual amino acids, it should be noted that the specificity of the method needs to be considered. The possibility exists that keto acids or other forms of the amino acids may be excreted and that they may stimulate the growth of the microorganisms as well as the amino acids themselves. Thus, the values reported on the excretion of any amino acid may be unduly high.

## SUMMARY

The excretion of 9 amino acids (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine) was determined in the urine of 4 normal and 4 undernourished subjects after the intravenous infusion of both physiological saline and an assayed amino acid mixture. The degree of undernutrition in the subjects ranged from mild to severe.

No difference was found in either the excretion of the individual amino acids or the total excretion pattern which could be attributed to the nutritional state of the subjects. Both groups retained approximately the same percentage of each of the administered amino acids.

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# THE INFLUENCE OF PTEROYLGLUTAMIC ACID AND VITAMIN $B_{12}$ ON THE METABOLISM OF PYRIDINE-FED RATS [1]

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THREE FIGURES

(Received for publication May 15, 1950)

The effects of dietary pyridine on rats have been recently reviewed by Baxter ('49). It was pointed out that, of the numerous supplements tested, only methionine afforded any reasonable degree of protection from pyridine toxicity. The literature contains numerous references which point to a metabolic relationship between vitamin $B_{12}$ and methionine (Jukes et al., '50; Oginsky, '50) and it seemed of interest to study the effects of $B_{12}$ on pyridine toxicity.

Data reported from this laboratory (Dinning and Day, '49a; Dinning et al., '49b, '50) indicate that pteroylglutamic acid (folic acid, PGA) is concerned in methylating processes, and for that reason a study was also made of its effect on pyridine-fed rats.

## EXPERIMENTAL

Seven groups of weanling Sprague-Dawley rats were fed the diets given in table 1. The rats were fed daily and any

[1] Research paper no. 907, Journal Series, University of Arkansas. This investigation was supported by a research grant from the National Institutes of Health, Public Health Service. The present paper was read before the Fifth Southwest Regional meeting of the American Chemical Society, Oklahoma City, December 8–10, 1949.

uneaten food was discarded the following morning. Food intake was restricted so that all groups consumed approximately the same quantity of diet. Body weight changes were determined for a 30-day growth period and complete peripheral blood counts were made between the 30th and 35th experimental days. Urine was collected for determination of creatine, creatinine (Folin, '14), and allantoin (Young and Conway,

TABLE 1

*Composition of experimental diets*

| DIETARY COMPONENTS | GROUPS | | | | | | |
|---|---|---|---|---|---|---|---|
| | I | II | III | IV | V | VI | VII |
| Casein (Labco) (%) | 15 | 15 | 15 | 15 | 15 | 15 | 15 |
| Sucrose (%) | 70 | 70 | 69 | 69 | 69 | 69 | 69 |
| Cod liver oil (%) | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Crisco (%) | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Salt mix[1] (%) | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| Pyridine (%) | 0 | 0 | 1 | 1 | 1 | 1 | 1 |
| PGA (gm/100 gm) | 0 | 0.5 | 0 | 0.5 | 0 | 0 | 0.5 |
| Liver ext. (units/100 gm) | 0 | 0 | 0 | 0 | 0 | 5 | 0 |
| $B_{12}$ ($\mu$g/100 gm) | 0 | 5 | 0 | 0 | 5 | 0 | 5 |

The liver extract used was an antipernicious anemia concentrate labelled to contain 15 units per milliliter. The $B_{12}$ concentrate was Squibb's "Rubramin." The following vitamins were added per 100 gm to all diets:

| | *mg* | | *mg* |
|---|---|---|---|
| Thiamine chloride | 0.5 | Choline chloride | 10.0 |
| Riboflavin | 0.5 | Pyridoxine hydrochloride | 0.5 |
| Nicotinic acid | 2.0 | Biotin | 0.005 |
| *i*-Inositol | 10.0 | Vitamin K | 0.025 |

[1] Hubbell, R. B., L. G. Mendel and A. J. Wakeman, J. Nutrition, *14:* 273, 1937.

'42). Liver choline oxidase was determined on rats sacrificed between the 30th and 35th experimental days. The liver was homogenized with 10 times its weight of 0.05 M phosphate buffer at pH 7.4. Five milligrams of choline chloride were added as substrate and oxygen consumption was measured for one hour. The results are reported as microliters of $O_2$ per milligram of dry weight per hour.

## RESULTS AND DISCUSSION

Average daily body weight changes for the 30-day growth period are given in figure 1. It may be noted that the pyridine reduced body weight gain and that this reduction was counteracted by either vitamin $B_{12}$ or injectable liver extract. These effects were statistically significant.[2] The addition of both PGA and vitamin $B_{12}$ to the basal diet seemed to suppress growth slightly. This suppression was most marked the last 7 days of the experiment. During this period the rats receiving the basal diet supplemented with both PGA and $B_{12}$ grew at

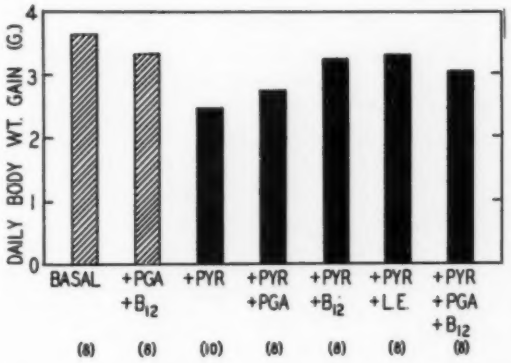


Fig. 1 Average daily body weight changes for rats receiving the various diets. The figures in parentheses refer to the number of rats.

only 75% of the rate of rats receiving the basal diet alone. Rats receiving the pyridine diet supplemented with PGA plus vitamin $B_{12}$ (group VII) grew at a normal rate for the first 20 experimental days. After that time their growth rate rapidly declined, and three rats of this group died between the 30th and 35th experimental days. On autopsy all rats in this group grossly exhibited pathological livers; these livers were yellow and infiltrated with fat. There were no deaths in any of the other 6 groups; similarly, in contrast to group VII, the livers

[2] The data were analyzed by the "t" test.

from the rats in these 6 groups appeared normal. These observations suggest that the rats in group VII were suffering from a choline deficiency. This may have resulted from an accelerated methylation of pyridine in the presence of PGA and vitamin $B_{12}$, with consequent loss of methyl groups from the body.

Table 2 presents data on the liver weights of rats receiving the various diets. The addition of pyridine to the basal diet increased the ratio of liver weight to body weight. Rats receiving the pyridine diet supplemented with PGA plus vitamin $B_{12}$ had the highest liver weight-body weight ratio of

TABLE 2

*The influence of PGA and vitamin $B_{12}$ on the liver weight-body weight ratio of pyridine-fed rats*

| | AVERAGE LIVER WEIGHT | |
|---|---|---|
| | *gm* | *% of body weight* |
| Basal | 8.49 | 4.20 ± 0.10 [1] |
| Basal + PGA + $B_{12}$ | 9.14 | 4.53 ± 0.14 |
| Pyridine | 8.02 | 5.54 ± 0.18 |
| Pyridine + PGA | 8.39 | 5.38 ± 0.20 |
| Pyridine + $B_{12}$ | 9.00 | 5.15 ± 0.23 |
| Pyridine + PGA + $B_{12}$ | 8.66 | 6.22 ± 0.14 |
| Pyridine + L.E. | 9.76 | 5.55 ± 0.11 |

[1] Standard error.

any of the groups. This is in agreement with the observation that the rats in this group exhibited evidence of fatty infiltration of their livers.

Kelley and Totter ('50) have reported that PGA exerted a lipotropic effect on rats when added to a purified diet containing 10% glycine. Thus it appears that PGA may exert a lipotropic or an anti-lipotropic effect, depending upon the experimental conditions.

Average values for creatine excretion per 100 gm of body weight are given in figure 2. There were no differences in the excretion of creatinine and allantoin among the rats re-

ceiving the various diets. It may be noted that PGA and vitamin $B_{12}$ increased creatine excretion when added to the basal diet. The increased creatine excretion by animals receiving the basal diet so supplemented would seem to be due to increased creatine synthesis, since none of the rats was losing weight. We have previously suggested that PGA functions in creatine formation (Dinning and Day, '49a). The addition of pyridine to the basal diet reduced creatine ex-

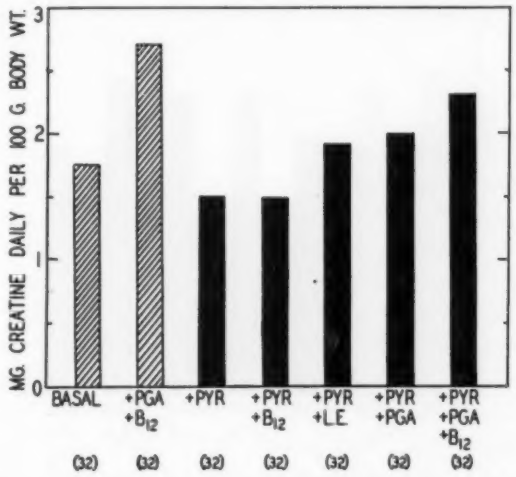


Fig. 2 Average daily creatine excretion per 100 gm body weight for rats receiving the various diets. The numbers in parentheses refer to the number of rat days from which the averages were taken.

cretion slightly. The addition of vitamin $B_{12}$ to the pyridine diet did not affect creatine excretion, while liver extract or PGA increased it. The addition of both PGA and vitamin $B_{12}$ to the pyridine diet (group VII) very markedly increased the output of creatine. This may be another manifestation of accelerated methylation in the presence of both PGA and vitamin $B_{12}$.

Average values for choline oxidase are given in figure 3. The addition of pyridine to the basal diet resulted in a re-

duced value for choline oxidase. The addition of either PGA, liver extract, or vitamin $B_{12}$ to the pyridine diet resulted in increased choline oxidase activity. The combination PGA plus vitamin $B_{12}$ did not increase choline oxidase when added to the pyridine diet; however, it has been pointed out that the livers from these rats were extremely fatty and it has been shown that fatty livers exhibit a reduced choline oxidase activity (Handler and Bernheim, '42).

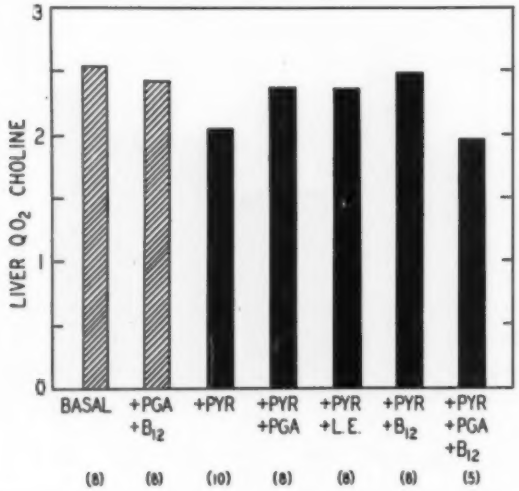


Fig. 3 Average liver choline oxidase values for rats receiving the various diets. The numbers in parentheses refer to the number of rats.

We have reported that aminopterin treatment inhibits liver, kidney and bone marrow choline oxidase (Dinning et al., '49b, '50), presumably by producing a PGA deficiency. The results reported in the present paper suggest that the feeding of either PGA or vitamin $B_{12}$ may increase rat liver choline oxidase.

There were no differences among the groups in red cell counts, hematocrit, hemoglobin, total white cell count and differential white cell count.

The mechanism whereby vitamin $B_{12}$ improves the growth of pyridine-fed rats is not clear. While pyridine is known to be detoxicated by methylation in some species, the methylated product has not been isolated from the urine of pyridine-fed rats (Baxter and Mason, '47). The effect of pyridine in reducing creatine excretion in these experiments may reflect a reduction in available methyl groups and could indicate that the pyridine was methylated. In this event, the effects of vitamin $B_{12}$ and PGA on these rats may have been due to a stimulation of methylating reactions.

## SUMMARY

Rats were given a basal purified diet containing 15% casein. The addition to this diet of 1% pyridine suppressed growth, creatine excretion, and liver choline oxidase activity. The addition of vitamin $B_{12}$ to the pyridine diet improved the growth of the rats, and the addition of either vitamin $B_{12}$ or pteroylglutamic acid resulted in increased liver choline oxidase activity. The addition of a combination of pteroylglutamic acid and vitamin $B_{12}$ to the diet of rats receiving 1% pyridine resulted in a greatly increased excretion of creatine; rats receiving this diet grew at a normal rate the first 20 experimental days but at a reduced rate thereafter. Three of the 8 rats receiving the pyridine diet supplemented with the combination of pteroyglutamic acid and vitamin $B_{12}$ died between the 30th and 35th experimental days; on autopsy the livers of all rats in this group were yellow and infiltrated with fat. There was no evidence of fatty infiltration in the livers of rats receiving any of the other diets. It is suggested that methylating reactions are stimulated in the presence of pteroylglutamic acid and vitamin $B_{12}$, resulting in an increased methylation of the pyridine. The excretion of this methylated pyridine would result in a deficiency of methyl, with the consequent production of fatty livers.

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# THE PREVENTION OF EXPERIMENTAL TOOTH DECAY BY TUBE-FEEDING

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EIGHT FIGURES

(Received for publication April 18, 1950)

Accumulation of food debris in the mouth has been suspected throughout the history of dental research to be a critical factor in the initiation and progress of tooth decay. Direct investigation of this problem is intrinsically impossible short of the complete elimination of such accumulations from the oral cavity throughout the full experimental period while other potential variables are held constant. Adequate nutriture of experimental animals without passage of food through their oral cavities appears to offer the surest, if not the only, procedure for the conduct of such an investigation. From a practical standpoint, there are three potential procedures for by-passing the mouth: by feeding through a stomach tube, through an esophageal or gastric fistula or by intravenous administration. In the present exploratory study, caries-susceptible rats have been maintained on a tube-feeding regimen where there has been essentially no contact of food with the oral cavity for periods as long as 25 weeks after weaning. Observations on the structure of the teeth and their supporting structures and on the initiation and development of carious lesions have been made for control and tube-fed rats.

## EXPERIMENTAL

The animals used in this investigation were offspring of rats which for two previous generations had been maintained on purified, caries-producing ration 100 (Shaw et al., '44). This dietary background has been shown to result in offspring with high susceptibility to tooth decay (Sognnaes, '48a). Prior to weaning, each litter and its mother were

TABLE 1

*Composition of the purified ration (204)*

| BASAL MIXTURE | | VITAMINS AND OTHER SUPPLEMENTS | | | |
|---|---|---|---|---|---|
| | *gm* | | | | |
| Powdered sugar [1] | 67 | Thiamine-HCl (μg) | 350 | Inositol (mg) | 100 |
| Blood fibrin | 24 | Riboflavin (μg) | 350 | *p*-aminobenzoic acid (mg) | 30 |
| Corn oil | 5 | Pyridoxine-HCl (μg) | 350 | β-carotene (mg) | 1.1 |
| Salt mixture [2] | 4 | Ca-pantothenate (mg) | 2 | Irradiated ergosterol (I.U.) | 300 |
| Whole liver substance | 2 | Nicotinic acid (mg) | 2.5 | α-tocopherol (mg) | 5.0 |
| 1:20 liver concentrate powder | 2 | Choline chloride (mg) | 100 | Menadione (mg) | 0.6 |

[1] Confectioner's sugar which contains 3% starch.

[2] Shaw, J. H., 1947, J. Dent. Res., *26*: 47.

maintained in a cage with wood shavings as bedding. Throughout the experimental period, the tube-fed and control rats were housed in screen-bottom cages.

The composition of the ration which was used in the present study is given in table 1 and will be referred to as ration 204. This diet, a slight modification of caries-producing ration 100, permitted a reasonably stable water suspension which flowed readily through a stomach tube with a fine bore. The control rats were allowed to consume this ration and

water ad libitum. Tube-fed rats received a suspension of ration 204 in the ratio of two parts of diet to one part of water. The mixture supplied 0.8 to 0.9 gm of dry ration (3.3 to 3.7 cal.) per milliliter. The water suspension was thoroughly mixed in a Waring Blendor just before each tube-feeding process.

For rats weighing up to 120 gm, a 20-ml Record syringe was used; for rats above this weight, a 50-ml Record syringe was more satisfactory. The small syringe could be held in a clamp on a ring stand and operated with one hand while the rat was held on its back in the other hand. The large syringe had to be held in a frame specially designed so that the plunger could be forced down by means of a threaded crank (fig. 1). The stomach tubes [1] used for small rats were about 10 cm long, while those for medium and large rats were in the neighborhood of 20 cm in length. The tube-feeding could be conveniently handled by one person for all sizes of rats.

The process of tube-feeding began by filling a Record syringe, attaching the stomach tube to the syringe, and almost filling the tube with the food mixture. Then, while the rat was held firmly on its back in the left hand with its mouth held open by a short length of rubber tubing, the

[1] The stomach tubes used in this investigation were made from polyethylene tubing which had an inside diameter of 0.047 inches and an outside diameter of 0.067 inches. These dimensions were found to be satisfactory for passage through the esophagus of recently weaned rats and for rapid delivery of the suspension of ration 204. The inelasticity of the polyethylene tubing made it necessary to form an adapter on the end of the tube which would fit over the metal tip of the Record syringe. This adapter was made by fusing a layer of polyethylene around the outside of the thin tube to form a shoulder about one inch long and roughly equivalent in its diameter to the tip of the Record syringe. A short length of rubber tubing whose inside diameter was the same as the outside diameter of the tip was slipped over the shoulder and fastened securely in place by a high melting point wax. The rubber tubing was allowed to extend beyond the open end of the tube to act as the connecting unit between the stomach tube and the syringe tip. Finally, to give sufficient rigidity to offset the elasticity of the rubber tubing during the feedings, the entire adapter assembly was bound with adhesive tape and a tightly wound spiral of wire.

polyethylene tube was guided gently along the hard palate through the esophagus to the stomach. An appropriate amount of food was introduced into the stomach at a rate of about 1 ml per second. Then the plunger of the syringe was retracted slightly to prevent any leaks of the food mixture into the oral cavity as the tube was removed from the esophagus. After the first two or three feedings, the rats seemed to become accustomed to the passing of the tube through the esophagus.

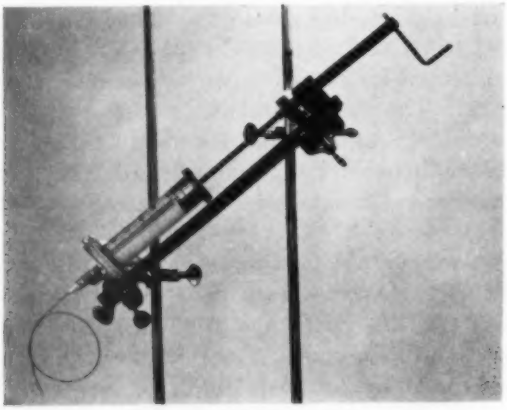

Fig. 1 The apparatus used for stomach-tube feeding of large rats consisted of a stand for a 50-ml Record syringe to the tip of which was attached the plastic stomach tube. The plunger is forced down by the threaded crank to eject measured amounts of the food suspension.

The quantity of food mixture fed to the rats was increased as they grew until the adult weight plateau was attained, in order to simulate normal rates of growth. Newly weaned rats (30 to 50 gm) were given 1 or 2 ml of the nutrient suspension for the first two feedings in order to accustom them to the procedure. After this, each rat was given 3 ml per feeding three times daily. As they grew, this quantity was gradually increased until the males received 7 to 8 ml per feeding and the females 5 to 6 ml per feeding throughout

the period of rapid growth. For a period of 4 weeks after the growth plateau was reached, two feedings per day were given of 8 to 10 ml each for males and 7 to 8 ml for females. For the last 4 weeks of the experiment, one feeding a day of 15 ml was given to males and of 12 ml to females.

The experiment began with 92 rats, 65 intact and 27 desalivated. Each experimental rat had a litter-mate control. The rats were placed on the experiment at weaning or shortly thereafter; the initial weights varied from 27 to 86 gm. Throughout the experimental period the rats were weighed twice a week. The experiment was planned to last 16 weeks. However, a preliminary examination of the control animals revealed a late occurrence of tooth decay. Therefore, the experimental period of the animals included in the data varied from 16 to 25 weeks.

The heads of the sacrificed animals were fixed in 10% formalin. Gross observations were made under a dissecting microscope. Caries was evaluated and recorded by the method of Shaw et al. ('44).

Microscopic sections were prepared of the jaws of representative litter-mate rats: one intact control and two intact experimental animals. Ground sections were prepared of a lower jaw by a plastic infiltration technique described elsewhere (Sognnaes, '47). Then the plastic embedding medium was removed from individual sections by immersion for 24 hours in methyl methacrylate monomer solution, whereupon the sections could be stained and mounted on glass slides. Paraffin sections of the other three quadrants were prepared after two and one-half weeks of low temperature decalcification in equal parts of 20% sodium citrate and 45% formic acid, a mixture recommended by Morse ('45). Deplasticized ground sections and deparaffinized, decalcified sections were stained with one or more of the following stains: hematoxylin-eosin, Masson, Gram and a 0.5% solution of toluidine blue in 5% alcohol.

## RESULTS

Before presentation of the main results, attention should be called to certain problems associated with feeding by stomach tube. No particular difficulty arose in connection with tube-feeding rats weighing 100 gm or more, i.e., in excess of 35 days of age. However, difficulties with recently weaned rats resulted in the death of a great number of the experimental animals. Thus, only 16 of 52 intact tube-fed rats and three of 23 desalivated tube-fed rats survived the experimental periods. Thirty-two deaths occurred in the first 5 days of the study; from the 6th to the 21st days there was an average of one death per day. This high rate of fatality was found to be largely preventable when the causes were recognized and avoided. No deaths occurred among either the 13 intact or the 4 desalivated rats which were allowed to eat ration 204 ad libitum.

The cause of death could be attributed to three factors. In the first place, the tube was apt to slip into the trachea of the younger rats. However, the esophagus permitted a smoother passage of the tube than the trachea, a fact which served as an important guide to the trained feeder. When the rats became larger and accustomed to the procedure, they seemed to assist by attempting to swallow the tube. Second, esophageal or gastric punctures occurred. These healed without damage when the tube was withdrawn before food was introduced, but death resulted if food was injected into the peritoneal cavity. Third, when excessive amounts of food were administered repeatedly, the rats developed a pylorospasm which resulted in death. This pitfall existed througout the experimental period. Animals so affected became dirty and sluggish and then developed an increasing abdominal swelling for one to three days before death. Autopsy revealed a stomach which was greatly distended with food and a tightly constricted pyloris. When tube-feeding was temporarily discontinued or greatly reduced at the onset of the above signs, the animal regained its normal condition in one or two days.

The average growth progress of those rats which completed the experiment is summarized in figure 2. In all groups of tube-fed animals the rate of growth was more rapid than for comparable controls. Desalivated animals grew at slower rates than intact animals when both groups ate ad libitum. However, tube-fed rats of the intact and desalivated series grew at comparable rates when fed similar amounts of food.

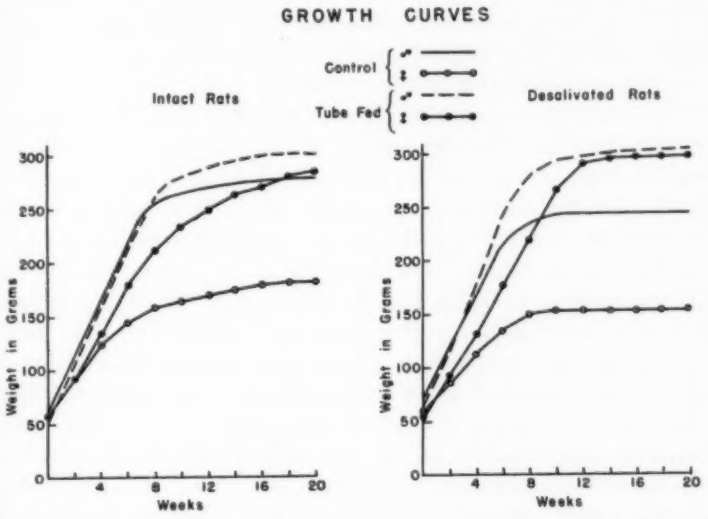


Fig. 2 Comparison between the rates of growth of tube-fed and litter-mate control animals.

The rates of growth of the tube-fed female rats, intact and desalivated alike, greatly exceeded those of control female rats and more nearly approximated the rates of growth of intact male rats. This probably indicated that somewhat greater amounts of food were administered to the tube-fed female rats than they would have eaten at will.

By gross examination it was possible to distinguish between the teeth of control and experimental animals. The nonoperated control animals had an irregular, diffuse brown-

ish stain over the visible tooth structure. The fissures were irregularly packed with dark debris. Very little tooth structure remained in the desalivated control animals; instead, there were granular, pulpy masses of tooth remnants. Intact and desalivated tube-fed animals had clean, white teeth free of foreign matter except for hard, discrete reddish or bluish impactions in the fissures. On the buccal surface of the maxillary teeth of the desalivated tube-fed animals was a heavy, yellowish, hard, brittle material which resembled calculus and which could be removed readily. The molar cusps of the experimental animals were somewhat less worn than those of the controls, but no difference was noted grossly in regard to periodontal structure and mobility of the teeth. Histological examination of the periodontal tissues confirmed the gross observation that comparable conditions existed in the supporting structures of the control and experimental groups.

The average incidence of tooth decay in the various groups is summarized in table 2. The values for male and female rats were averaged together because unpublished data from this laboratory on a total of about 3,000 cotton rats and Long-Evans and albino strains of the Norway rat indicated that there was no sexual difference in dental decay under these experimental circumstances. All the 13 tube-fed intact and the three tube-fed desalivated rats were free of tooth decay. On the other hand, the 13 intact controls had an average of 5.0 carious molars, 6.7 carious lesions and an average caries score of 10.6 +; the 4 desalivated control rats had an average of 11.5 carious molars, 28.8 carious lesions and an average score of 124.8 +. Statistical evaluation of the differences in number of carious molars, number of carious lesions and caries score between control and experimental groups indicated that a highly significant reduction in tooth decay had been produced in intact and desalivated rats by tube-feeding; the critical ratios ranged from 5.7 to 26.6.

Tooth decay in rats raised under a regimen comparable to that of our intact control animals has already been studied

TABLE 2

*The incidence of tooth decay in rats fed by stomach tube and in litter-mate controls*

| CONDITION | METHOD OF FEEDING | NO. OF RATS | NO. OF CARIOUS MOLARS | | | NO. OF CARIOUS LESIONS | | | EXT. OF CARIOUS LESIONS | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Average | S.E.M.[1] | C.R.[2] | Average | S.E.M.[1] | C.R.[2] | Average | S.E.M.[1] | C.R.[2] |
| Intact | Normal | 13 | 5.0 | 0.7 | > 7.0 | 6.7 | 0.9 | > 7.7 | 10.6 + | 1.6 + | > 6.5 |
| | Stomach tube | 13 | 0 | 0 | | 0 | 0 | | 0 | 0 | |
| Desalivated | Normal | 4 | 11.5 | 0.4 | > 26.6 | 28.8 | 3.5 | > 8.3 | 124.8 + | 21.6 + | > 5.7 |
| | Stomach tube | 3 | 0 | 0 | | 0 | 0 | | 0 | 0 | |

[1] S.E.M. — Standard error of mean, the chance variability of the mean.

[2] C.R. — Critical ratio, the value which indicates the degree of relationship between two groups. When this ratio is greater than three, the difference is considered to be statistically significant.

histologically and could not be distinguished from caries in man (Sognnaes, '48a, b). In the present report, we have been primarily concerned with the histological character of the molars and of the material impacted in their occlusal fissures. The following illustrations are, therefore, limited to sections of the molar teeth of intact, experimental and control rats which were sacrificed before the appearance of advanced lesions: namely, after 19 experimental weeks.

The ground sections shown in figures 3 and 4, which have been stained with toluidine blue, represent molars of control and experimental animals, respectively. This dye had a strong affinity for the enamel of the molars of the control rat (fig. 3) but the enamel of the tube-fed rat was practically unstainable (fig. 4), which is indicative of a high degree of mineralization of the organic framework of the enamel. Especially at higher power, the enamel of the molars in the control rat exhibited the metachromatic color reaction in the interprismatic regions which, according to Wislocki and Sognnaes ('49, '50), is characteristic of the enamel of unerupted and immature erupted teeth. This difference in the degree of mineralization between the enamel of the two groups was also borne out by examination of the ground sections in polarized light.

The nature and exact location of the foreign materials impacted in the fissures of the molars could be most satisfactorily examined by means of paraffin sections, some of which are shown in figures 5 to 8. It is of considerable interest that an abundance of microorganisms could be found in sections from the molar teeth of tube-fed and control animals alike. In sections of the control animal, shown in figures 5 and 7, the dark-staining (basophilic) microorganisms had infiltrated and surrounded the lighter staining bits of wood shavings which, as already mentioned, were frequently encountered in the molar fissures of both groups. However, in the tube-fed group the microorganisms seemed, in the first place, to have had a lesser tendency to infiltrate and surround the foreign matter in the molar fissures, as is indicated by

figure 6. Secondly, even if the molar fissures of the tube-fed group happened to be free from impacted matter, it was found that a fairly heavy, transparent membrane separated the invading microorganisms from the enamel surface. This membrane can be seen in figure 8 as a lightly stained zone between the dark staining microorganisms in the fissure and the empty space formerly occupied by the enamel. (The highly calcified enamel has been lost during the process of histological preparation.) This membrane stained green with toluidine blue, greyish-red with Masson's stain, and evenly red with Gram stain. In protected areas, an occasional epithelial cell or remnants thereof could be found. In view of these cellular remnants and because of the staining reaction which appeared very similar to that of keratinizing surface epithelium in the same sections, it is possible that the separating membrane represents remnants of the so-called Nasmyth's membrane (Nasmyth, 1839).

## DISCUSSION

Previous investigations (Sognnaes, '48a, b) have demonstrated that the carious lesions in common laboratory rats maintained under the same dietary circumstances as the controls in this experiment were comparable to human caries, and that these lesions were not preceded by traumatic or mechanical injury to the enamel. Hence, the prevention of tooth decay in the tube-fed rats of the present experiment cannot be attributed to the elimination of mechanical injury as the initiating factor.

Caloric restriction has been demonstrated to result in significant reductions in the initiation and development of carious lesions in caries-susceptible cotton rats and white rats (Shaw, '48, '50). Since the average rates of growth of the tube-fed rats in all cases were at least as rapid as those observed in comparable control rats, it is obvious that the caries reduction associated with tube-feeding cannot be explained by caloric restrictions.

Removal of the principal salivary glands has been found to increase greatly the caries incidence of rats maintained even on a less caries-producing diet than the one used in this experiment (Weisberger, Nelson and Boyle, '40). It is, therefore, particularly significant that tube-feeding resulted in prevention of tooth decay in spite of the penalty of desalivation.

Complete absence of masticatory function due to extraction of opposing molars has been shown to result in marked changes in the supporting structures of rats' teeth (Sognnaes, '41). In view of the different mode of food intake and presumably the difference in masticatory function between our tube-fed and control groups of animals, it is worthy of note that no significant difference was found in the supporting structures of the molars: the bone, periodontal membrane and cementum. One wonders to what extent the tube-fed animals may have exercised their jaws and molars even though no masticatory function was required for digestive purposes.

The difference in stainability of the undecalcified ground sections is believed to be a reflection of the degree of maturation of the enamel. Mature intact enamel in adult animals is normally quite inaccessible to histological and histochemical stains (Wislocki and Sognnaes, '49; Sognnaes and Wislocki, '50). This was true of the enamel of the molars in the tube-fed rat, shown in figure 4. An abnormally great affinity for stain was evident in the enamel of the normally fed animal (fig. 3), and yet the rats were litter mates which were sacrificed the same day, at which time they were almost identical in weight. In the control group, the enamel of the teeth exposed to the oral environment containing the purified ration either had failed to reach the same degree of maturity that had been reached by the enamel of the tube-fed animals, or, once mature, the teeth had been demineralized to a point where the organic matter again became accessible to stains. The former possibility is suggested by the location of the stain in the cervical and occlusal parts of the enamel, which were the most recently formed. In either case, it would

seem that this abnormality, the incomplete calcification of the organic framework, may represent one step closer to decay.

The Nasmyth's membrane or the "skin of the teeth" is generally supposed to be worn off soon after eruption of the teeth. In the tube-fed animals, remnants of a similar membrane were nevertheless retained in the adult molar teeth. It is reasonable to assume that the membrane has been retained because of the lack of physical wear or because of differences in the chemical and bacterial environment concomitant with the absence of food in the mouth.

The oral environment was not subjected to chemical or bacteriologic tests in this study. Histologically, microorganisms were found around the teeth in both groups whether the purified ration was taken into the mouth or not, except that the above-mentioned membrane and the prolonged retention and denser impaction of inert foreign matter (wood shavings) seemed to act as a separating medium between the enamel surface and the microorganisms. The wood shavings which were present in the molar fissures of both the tube-fed and control rats must have been forced into these areas during the pre-experimental period, and retained there throughout the experiment. The teeth of the tube-fed animals, therefore, seemed to distinguish themselves from those of the controls not only by the absence of caries, but by the lack of stainability of the enamel and by the lack of a direct contact with the microorganisms.

Pre-experimentally, the animals used in this study had been conditioned by the dietary regimen during their prenatal and early post-natal life in such a way that their teeth were generally susceptible to tooth decay. But the end results of this experiment establish satisfactorily that, even under these experimental conditions, food had to be present in the oral cavity in order for carious lesions to develop in the caries-susceptible teeth. The procedure of tube-feeding should

be well suited for further detailed investigations on the etiology of tooth decay, on the rate and process of maturation of the enamel of the teeth, and on the progress of carious lesions when the oral environment is experimentally altered.

## SUMMARY

1. When intact or desalivated caries-susceptible albino rats were fed adequate amounts of a caries-producing diet by stomach tube they did not develop tooth decay. In contrast, all but one of the intact litter mates and all of the desalivated litter mates which consumed the same diet orally had dental caries. The critical ratios of the differences between the control and experimental groups ranged from 5.7 to 26.6, which indicated that the differences were highly significant.

2. Histologic examination showed that the molar teeth of both groups were surrounded by a great abundance of microorganisms. In the controls, the microorganisms were in direct contact with the tooth surfaces; in tube-fed rats they were separated from the enamel surfaces by a thick organic membrane (possibly remnants of Nasmyth's membrane) and by foreign matter which was firmly impacted in the fissures.

3. The results of this experiment suggest that, with all other factors being controlled and equal, tooth decay is prevented in caries-susceptible rats when the direct effects of food in the oral cavity are eliminated.

## ACKNOWLEDGMENTS

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## PLATE 1

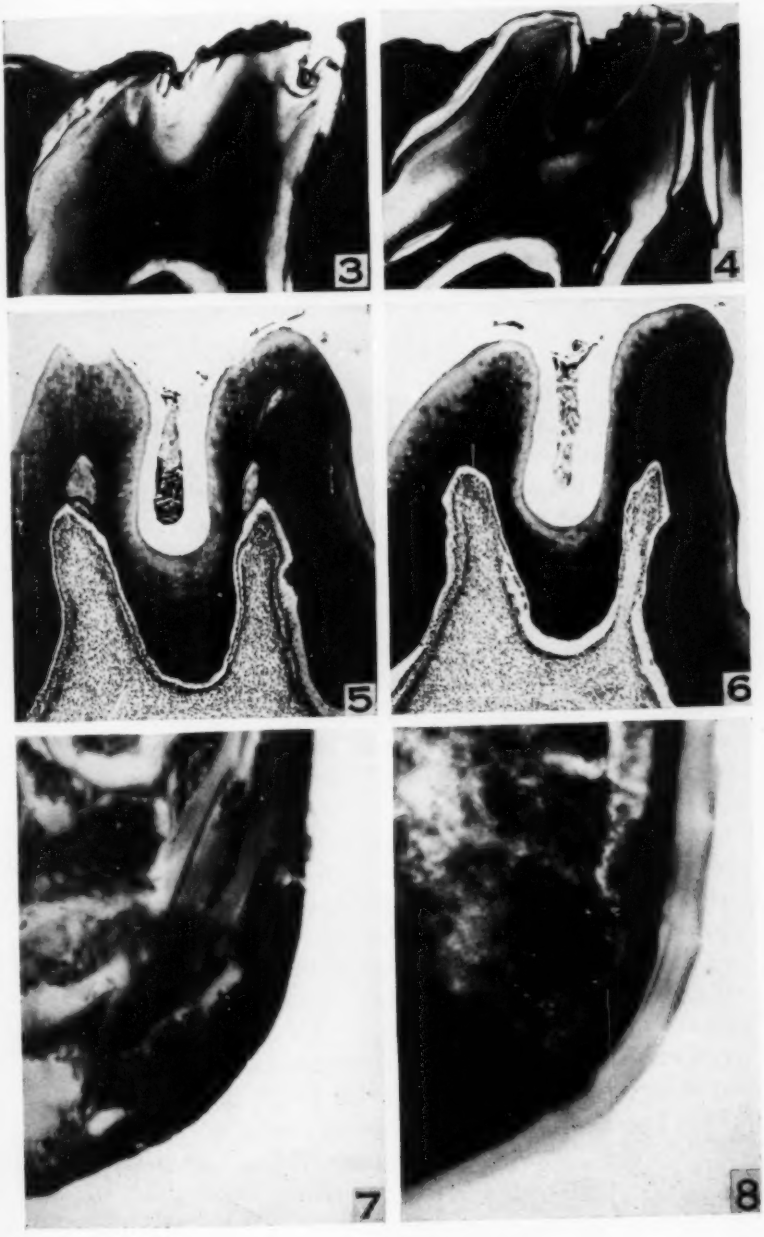
### EXPLANATION OF FIGURES

3 and 4 Undecalcified sections of molar teeth, showing that the enamel of the tube-fed animal (fig. 4) has taken up less stain than that of the control (fig. 3), indicating a higher degree of mineralization of the organic framework in the former. (Plastic embedded ground sections. Toluidine blue stain. × 20.)

5 and 6 A light-staining foreign matter (wood shavings) is seen in the occlusal fissures of the molars of both the control (fig. 5) and tube-fed animal (fig. 6). But in the control these impactions are surrounded by a dark-staining (basophilic) material which is in direct contact with the enamel. (Decalcified paraffin sections. Toluidine blue stain. × 40).

7 At this higher power of the fissure shown in figure 5, the basophilic material is seen to consist of minute dots which could be identified as predominantly Gram-positive spheroid microorganisms. These surround the paler, impacted matter and are in direct contact with the space formerly occupied by the enamel. (As in figs. 5 and 6. × 800.)

8 Higher magnification of a molar fissure of a tube-fed rat. In this case, the bottom of the fissure is free from impacted wood shavings and instead is filled by a great abundance of microorganisms. However, in contrast to the control shown in figure 7, these organisms are seen to be isolated from the enamel by a thick semitranslucent membrane, which is believed to be a remnant of Nasmyth's membrane. (Decalcified paraffin section. Toluidine blue stain. × 800.)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# THE EFFECT OF FLUORIDE AND GRAPEFRUIT JUICE ON THE ETCHING OF TEETH

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THREE FIGURES

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Recent experiments indicate that the hydronium ion is an important factor in the decalcification of teeth and in the subsequent exposure of the dentin layer. The hydronium ion may be furnished *in situ* through the action of the *Lactobacillus Acidophilus* flora of the buccal cavity, causing the formation of acids from food starches and sugars retained between the teeth. Kesel ('48) has reported the carious areas to be more acid than the non-carious and the *Acidophilus* count to increase during active caries. He has also called attention to the lowering of caries' incidence in the Scandinavian countries during the Second World War, when sugar intake was limited by rationing.

The concentration of the hydronium ion can be increased also by the intake of acidic foods and liquids. Wynn and Haldi ('48) have found that liquids with a pH of 5.4 or lower aid in the decalcification of teeth. For example, moderate etching has been obtained in one and one-half weeks with an acetic acid-sucrose solution (pH 2.6), and within two weeks with orange juice (pH 3.7). On the other hand, solutions of phosphoric acid and of lactic acid with a pH of 4.5 apparently have had no effect. Several beverages and fruit juices have been found to be causative agents of dental caries (Bieri, McCay, Restarski and Gortner, '46).

Proper buffer action of the saliva could prevent the dissolving effect of the strong acid solutions on the teeth. McCay and Will ('49) measured the buffer capacity of the saliva in a group of people and found a considerable number who failed to have such buffer action and who had, therefore, conditions which would lead readily to the erosion of teeth.

Recent attempts have been made to provide a protective coating over the teeth in an attempt to diminish the contact of acid substances with the enamel. McCay and Will ('49) have reported that oxalic acid dissolved in an acid beverage deposits effective layers on the teeth, but that sodium oxalate ingested with the food is less effective.

Considerable evidence has accumulated that sodium fluoride, in solid or liquid foods, is effective in decreasing the incidence of caries. The fluoride ion seems to be absorbed and preferentially stored in the enamel layer (Phillips and Muhler, '47), where it contributes to greater hardness and to greater resistance to erosion.

Because of the public health interest in the fluoride treatment of dental caries, it was thought desirable to study the effect of sodium fluoride in preventing the erosive action of acid beverages. It was decided to use grapefruit juice since: (1) it has been found to have an erosive action; (2) it is a common household fruit juice; and (3) no study of the effect of added fluoride had been reported at the beginning of this experiment. Sucrose was added to the juice, since Wynn and Haldi ('48) had found it necessary for palatability. The total sucrose content was within the range of other commercial fruit juices such as grape juice and cranberry juice cocktail.

## EXPERIMENTAL

A study of the extent of tooth erosion following the drinking of grapefruit juice and of the degree of inhibition provided by the addition of the fluoride ion was made by the use of litter-mate, Sherman stock, male rats. In the first series, hereafter designated series 1, each of three groups of 4 males was given one of the following liquids as the sole source of

fluid to drink: distilled water; commercially canned grapefruit juice to which had been added 10% by weight of sucrose; and canned grapefruit juice from the same case lot to which had been added 10% sucrose and 50 p.p.m. of the fluoride ion, as sodium fluoride. In the second series, series 2, employing the same number of males per group, the three groups of series 1 were repeated with two additional groups, each given one of the following as the sole source of liquid: distilled water to which had been added 10% sucrose by weight; and grapefruit juice, with 10% sucrose, neutralized with U.S.P. sodium bicarbonate, using brom thymol blue as the indicator. The male rats in each of the groups were about 4 weeks of age and were matched as nearly as possible in weight. Three of the experimental animals subsequently died of colds.

The animals were fed a locally prepared pellet feed dispensed ad libitum in open cups. The liquid, previously described, was provided in drinking tubes to minimize contamination and evaporation. Records were kept of the liquid consumption and an attempt was made to keep it the same per rat per day. This proved difficult in series 2, since the sugar-water animals often consumed their day's allotment within the first hour after feeding. Fresh liquid was given daily except over Sunday.

Ten per cent sucrose, U.S.P., was added to the grapefruit juice to bring the total sugar content to about 20% for palatability. The sugar content was high but within the range of other canned fruit juices. The concentration of 50 p.p.m. of the fluoride ion was chosen because it had been reported to be about 80% effective by McClure ('41) and probably below the level associated with dental fluorosis.

After 11 weeks had elapsed, the experimental animals were chloroformed and decapitated. The heads were boiled for about two hours in distilled water. The lower jaws were then easily removed from the skull and the jaws and teeth cleaned with a fine pick. Only the lower molars were considered in this experiment, since they had the greatest con-

tact with the fluids in the mouth. The teeth were examined under a wide field microscope.

The teeth were examined for their degree of erosion in terms of the scale devised by Restarski, Gortner and McCay ('45), which is as follows:

0 — no effect;
1 — high polish of the lingual enamel;
2 — slight etching of the lingual enamel;
3 — mild destruction of the lingual enamel with evidence of slight ridge formation at the gingival margin;
4 — moderate destruction of the lingual enamel with more definite ridging and some exposure of dentin;
5 — severe destruction of the lingual enamel with marked ridging at the gingival margin and appreciable exposure of the dentin;
6 — almost complete destruction of the lingual enamel with definite evidence of destruction on other surfaces, and marked exposure of the dentin with some destruction.

The pH of the fluids was determined by means of the glass electrode. Samples were selected which had been stored in the laboratory under conditions similar to those to which the liquids being consumed were exposed. All of the grapefruit juice solutions except the neutralized grapefruit juice were below the pH specified as necessary for corrosion.

## RESULTS AND DISCUSSION

Good growth was obtained on the pellet diet with or without grapefruit juice solutions as the source of liquid. The average gain in weight per rat over the 11-week period was 187 gm. The gain in weight for each of the groups can be seen in table 1. The animals on the neutralized grapefruit juice were the only ones to show poor growth, although their liquid consumption was equal to that of groups gaining almost three times as much during the same period. The growth of this particular group was erratic, fluctuated between gains and losses, and was attended by sporadic signs of diarrhea.

The fluid consumption in series 1 averaged from 25 to 27 ml per rat per day for each of the three groups. Individual cases showed variations of from 14 to 36 ml per day. In series 2 the fluid consumption was slightly less, i.e., it averaged from

18 to 22 ml per rat per day. The slight variation in fluid consumption probably had no significant effect upon the teeth, since McClure ('43) has found the pH of the solution to be more important in leading to corrosive action than the amount of liquid imbibed. Differences in season and room temperature probably accounted for the difference between the two series.

TABLE 1

*Growth and fluid intake of rats on grapefruit mixtures*

| LIQUID | NO. IN GROUP | AVERAGE INITIAL WEIGHT | GAIN IN 11 WEEKS | LIQUID INTAKE PER RAT PER DAY |
|---|---|---|---|---|
| | | *gm* | *gm* | *ml* |
| Distilled water | | | | |
| Series 1 | 4 | 56 | 230 | 25 |
| Series 2 | 3 | 57 | 238 | 22 |
| Distilled water and 10% sucrose | | | | |
| Series 2 | 4 | 57 | 173 | 20 |
| Canned grapefruit juice and 10% sucrose | | | | |
| Series 1 | 4 | 55 | 198 | 27 |
| Series 2 | 4 | 57 | 173 | 18 |
| Canned grapefruit juice, 10% sucrose and 50 p.p.m. fluoride | | | | |
| Series 1 | 4 | 55 | 230 | 25 |
| Series 2 | 2 | 56 | 185 | 22 |
| Canned grapefruit juice, 10% sucrose, neutralized | | | | |
| Series 2 | 4 | 60 | 67 [1] | 20 |

[1] In the neutralized grapefruit juice group, maximum gain in weight rather than final gain in weight is given since there was consistent loss in weight the last 4 weeks of the experiment.

The teeth of the animals in the control group, consuming pellets and water, appeared to be without erosion. On the grapefruit juice the teeth of the rats varied in appearance from mild destruction of the lingual enamel with ridging at the gingival margin to moderate destruction of the lingual enamel with more definite ridging and some exposure of the dentin. The erosion appeared where there was direct contact with the fruit juice. The roots of the teeth, protected by the gingiva, appeared to be in perfect condition.

The different effects of the three solutions can be seen in plate 1. The jaws were selected from three litter mates from each of the three experimental groups in series 1. There appears to have been a greater protective action of fluoride in one animal (no. 3) than was seen microscopically for the group as a whole. The individual scores can be seen in table

TABLE 2

*Erosive effect of grapefruit juice mixtures on lower molar teeth*

| LIQUID | RESTARSKI SCORE [1] | | pH OF LIQUID |
|---|---|---|---|
| | Series 1 | Series 2 | |
| Distilled water | 0 | 0 | 6.7 |
| | 0 | 0 | |
| | 0 | 0 | |
| | 0 | | |
| Distilled water, 10% sucrose | | 0 | 6.6 |
| | | 0 | |
| | | 0 | |
| | | 0 | |
| Canned grapefruit juice, 10% sucrose | 3 | 3½ | 3.8 |
| | 4 | 3½ | |
| | 3 | 4 | |
| | 4 | 3½ | |
| Canned grapefruit juice, 10% sucrose, 50 p.p.m. fluoride | 2½ | 2 | 3.7 |
| | 2 | 2 | |
| | 2½ | | |
| | 2 | | |
| Canned grapefruit juice, 10% sucrose, neutralized | | 0 | 6.6 |
| | | 0 | |
| | | 0 | |
| | | 0 | |

[1] See text, page 110.

2. While the number of cases in each group was not large, the close parallelism between the two series within the same group as to the degree of erosion would seem to indicate that the results were conclusive.

Erosion of the teeth was observed only on diets containing grapefruit juice at pH's of 3.7 and 3.8. The sucrose added to

the grapefruit juice was apparently not contributing to the etching, since no erosive effect on the teeth was found with sucrose and distilled water. However, there were extensive caries present on the sugar-water fluid, so that acids produced from sugar would seem to induce caries but not generalized erosion.

Factors other than sucrose and acidity, present in grapefruit juice, were also non-contributory to corrosion, since no etching was observed in teeth coming in contact with neutralized grapefruit juice. These results confirm the findings of previous reports that beverages with a pH below 5.4 cause erosion of teeth.

A moderate protective action against etching was found when the fluoride ion, added as sodium fluoride, was ingested with the grapefruit juice at a level of 50 p.p.m. The appearance of the teeth varied from slight etching to mild destruction of the lingual enamel. There was evidence of slight ridge formation at the gingival margin, but no exposure of dentin. There seemed to be about 60% protection against the etching effect by acids at this fluoride level. Whether complete protection could be obtained by the use of higher levels of the fluoride ion, and whether such a level would be so high as to induce fluorosis, could not be predicted on the basis of the present study. With respect to dental caries, it has been found that 50 p.p.m. of the fluoride ion in drinking water is 80% effective against their development, and 125 p.p.m. gives practically complete protection (McClure, '41).

The erosive effect of the juice would probably have been more extreme if the experiment had been continued for two or three more weeks. During the course of the experiment extra animals were given grapefruit juice and killed two and 5 weeks earlier than the experimental groups. Since little erosion was found to have occurred at this time, it appears that the major part of the erosion in the experimental group had occurred in the last two weeks, and therefore that such erosion was not a linear function.

SUMMARY

The lower molars of young male rats were found to be strongly etched after the animals drank commercially canned grapefruit juice as the sole beverage during an 11-week period. The erosion was due to the acidity and not to the sugar content or any other factors, since no erosive action was found when sucrose-water or neutralized grapefruit juice became the fluid intake. There was about 60% protection when 50 p.p.m. of the fluoride, as sodium fluoride, were added to the grapefruit juice.

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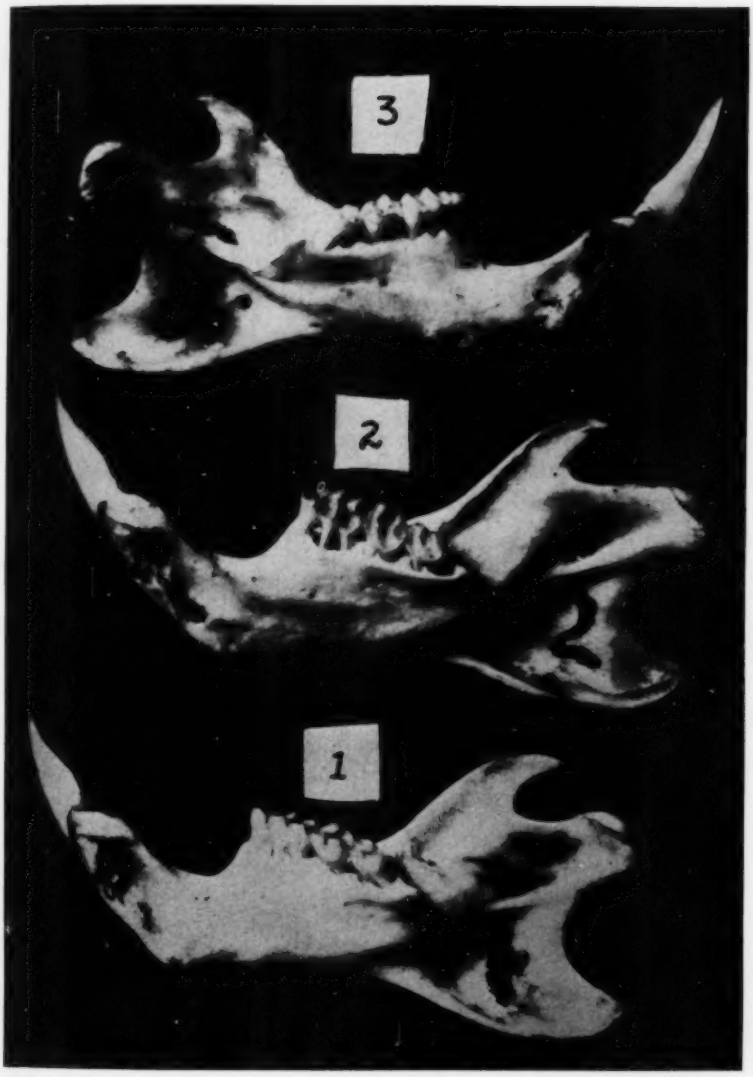


Fig. 1 (Bottom) Lower molar teeth with distilled water as beverage.
Fig. 2 (Middle) Lower molar teeth with grapefruit juice as beverage.
Fig. 3 (Top) Lower molar teeth with grapefruit juice plus 50 p.p.m. fluoride as beverage.

# CONTRIBUTIONS OF BREWERS' YEAST TO A DIET DEFICIENT IN REPRODUCTIVE FACTORS[1]

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Dried brewers' yeast has nutritional importance as a feed supplement because it has a high protein content and because it supplies most, if not all, of the B vitamins. Brewers' yeast has been studied for its effect on various rations as a protein supplement, as the sole source of protein, as a B vitamin supplement and as the sole source of B vitamins. However, the reports of these studies in the literature are both scattered and contradictory.

Osborne and Mendel ('19) fed rats on brewers' yeast as the sole source of protein. Those on 40% levels were maintained with a normal growth rate for more than a year, but those on a 30% level showed rather poor growth during this period. Nelson, Heller and Fulmer ('23) succeeded in rearing three generations of rats on a diet containing 45% dried brewers' yeast; however, the growth of the offspring was always slightly below normal and lactation was poor. Sure ('46) fed a brewers' yeast at 30% and 40% levels and noted that the growth, reproduction and lactation performances in three consecutive generations of rats were comparable to those of rats on a diet containing 15% purified casein. In subsequent studies with another type of brewers' yeast, how-

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by a grant from the Brewers' Yeast Council, Inc., St. Louis, Missouri. We are indebted to Merck and Company, Inc., Rahway, New Jersey, for crystalline vitamins and vitamin $B_{12}$ concentrate.

ever, he communicated to José Goyco that he was unable to obtain even normal growth.

Goyco and Asenjo ('48) compared the growth, reproduction and lactation of rats fed various levels of brewers' yeast as the sole source of protein with those of their stock colony rats. Reproductive performance was always inferior to that of the stock colony rats. Only those diets containing 30% or more of yeast gave second generation rats. At the 40% level 4th generation animals were obtained with normal reproduction but poor lactation, as was shown by the weight of the 28-day-old weanlings, which was only one-half as much as that of the stock colony weanlings.

Lactation may be the function most adversely affected by an inadequate diet and, as might be expected, brewers' yeast was more often reported to be inadequate or borderline in support of this function than in support of growth or reproduction.

Bowland, Ensminger and Cunha ('48), using a purified ration supplemented with 10 B vitamins, reported the need of an additional factor or factors for reproduction and lactation. Both Vinson and Cerecedo ('43) and Richardson and Hogan ('45) maintained rats on highly purified, vitamin-fortified rations and obtained growth and reproduction equivalent to those of the stock colony animals. However, in both cases lactation was subnormal, with only 60 to 70% of the young weaned. Vinson and Cerecedo ('44) also reported that the mothers lost up to 30% of their body weight during lactation but that daily supplementation with 0.5 gm of brewers' yeast powder [2] prevented this weight loss.

More recently Nelson and Evans ('47) were able to obtain 80 to 90% weaning and moderately good weaning weights on a purified ration by improving the salt mixture and by supplementing with 8 B vitamins. Although this is the best lactation record yet obtained on a highly purified ration, the suboptimum weaning weights of the young and a loss of weight

[2] Strain K, from Anheuser-Busch, Inc.

by the lactating mothers still point to an additional dietary requirement for optimum lactation.

Evans and Burr ('28) reported that brewers' yeast, fed as a source of B vitamins with a partially purified diet, was required at a level of 15% in order to support normal lactation in the rat and to produce normal weight in the young.

Nakahara and Inukai, in a series of papers ('33, '36, '40) reported the need of lactation factors $L_1$ and $L_2$, and found brewers' yeast to be a good source of both. Cunha ('44) repeated Nakahara's preparation of $L_1$ and $L_2$ from fried brewers' yeast but failed to find a lactation-stimulating effect therein.

Ross, Phillips and Bohsted ('42a, b) and Cunha, Ross, Phillips and Bohsted ('43) found that a corn-soybean oil meal-alfalfa ration supported growth and maintenance in swine and rats but was incapable of supporting successful reproduction and lactation in these species. The addition of 5% brewers' yeast to this ration reduced sterility considerably and increased the weaning rate in rats to 74%.

Cunha ('44) found brewers' yeast to be a borderline carrier of the factor or factors needed to supplement this corn-soybean oil meal-alfalfa feed, and Spitzer and Phillips ('46) reported variable effects on reproduction and lactation when the ration was supplemented with brewers' yeast.

## EXPERIMENTAL

The purpose of this series of experiments was to determine the contribution of brewers' yeast when used as a supplement to a practical corn-soybean oil meal-alfalfa ration. The effect on reproduction and lactation in rats was studied when the basal ration was supplemented with various brewers' yeasts, liver, fish solubles and vitamin $B_{12}$.

The composition of the basal ration was as follows: yellow corn meal 75.3%, soybean oil meal (expeller-processed) 17.5%, alfalfa meal 5.0%, $CaHPO_4$ 1.0%, $CaCO_3$ 0.65%, iodized NaCl 0.50%, and $MnSO_4$ 0.01%, plus two drops of haliver oil per rat per week.

Except in the first experiment, female albino rats of the Holtzman strain were separated into groups comparable in numbers and weights. Four-month-old animals were used in experiment 2 and rats two and one-half months old in experiment 3. Each group was standardized by a depletion period of three to 5 weeks before being started on the basal ration plus the test supplement. After depletion of the females, three to 4 female rats and one stock colony male were placed in a group cage having a false bottom of wire mesh. Once a week the male rat was replaced by another which had been on the stock ration for at least two weeks. The females were kept in the group cages for 12 weeks in the preliminary experiment, and 6 weeks in each of the following two experiments. Food and water were fed ad libitum. On detection of pregnancy, the female rat was removed to an individual cage without a false bottom and given wood shavings as a nesting material.

The mothers were left undisturbed for 48 hours after parturition, at which time they and the pups were weighed, the pups counted and the litters reduced to a standard size of 6 pups each. This was done in order to have a comparable basis for measurement of success in lactation. The pups were weaned at 21 days. The mothers were rested for three weeks before being returned to the mating cages for a second 6-week period, during which their diet contained 5% fish solubles in addition to their previous supplement.

## RESULTS AND DISCUSSION

### *Experiment 1*

The preliminary experiment was conducted with female rats of assorted ages and dietary histories. A 5-week depletion period did not nullify the effect of the former rations, but it was nevertheless evident that neither dried brewers' yeast S at 6% nor dried brewers' yeast A at 6% increased the success of reproduction and lactation. The results are summarized in table 1.

*Experiment 2*

The second experiment was designed to test 6 other brewers' yeasts.[3] In order to minimize the possibility of toxicity due to high levels of purines, the yeasts were fed at 3% rather than at 6%. Two of the samples, 2 and 5, were also fed at 6%, with no evident difference in performance by the rats. The data for both levels of the same supplement are therefore recorded together without differentiation. Table 2 presents a summary of the results of these experiments.

TABLE 1

*Effect of certain brewers' yeasts on reproduction in the rat*

| CATEGORY OF INTEREST | YEAST SUPPLEMENT | | |
|---|---|---|---|
| | None | 6% S | 6% A |
| No. females | 5 | 7 | 8 |
| No. sterile | 2 | 3 | 3 |
| No. pups born | about 12 | about 38 | about 29 |
| No. pups weaned | 5 | 14 | 5 |
| Per cent weaned | 42 | 37 | 17 |

None of the yeast supplements fortified the ration for the successful support of reproduction and lactation. Only one litter out of the total of 22 born survived until weaning. Approximately two-thirds of the pups were eaten by the mothers, usually within the first two days, and, of the others, many were found dead but uneaten. At 48 hours only 14% of the pups were still living, and of these only three pups were weaned.

The addition of 5% fish solubles to the 6% yeast supplement (at the expense of the whole ration) caused marked improvement in the reproductive performance of these rats.

[3] These were: 4 samples from the St. Louis Brewers' Yeast Company, no. 1 — no. 4; one from the Brewers' Yeast Council, no. 5: and a Budweiser sample, no. 6.

TABLE 2

*Reproductive performance of rats fed diets supplemented with brewers' yeast only, and with brewers' yeasts plus fish solubles*

| YEAST SUPPLEMENT | NO. OF FEMALES | | NO. OF LITTERS | | AVE. NO. OF PUPS PER LITTER | | AVE. WEANING WEIGHT PER RAT |
|---|---|---|---|---|---|---|---|
| | Bred | Sterile | Lost | Weaned | Born | Weaned [1] | |
| | | | | | | | *gm* |
| Basal ration + 3% no. 1 | 4 | 1 | 2 | 1 | 7.0 | 3 | 28.4 |
| Basal ration + 3% no. 2 | 3 | 1 | 2 | 0 | ? | 0 | ... |
| Basal ration + 6% no. 2 | 3 | 0 | 3 | 0 | 6.0 | 0 | ... |
| Basal ration + 3% no. 3 | 4 | 2 | 2 | 0 | 5.5 | 0 | ... |
| Basal ration + 3% no. 4 | 4 | 1 | 3 | 0 | 7.0 | 0 | ... |
| Basal ration + 3% no. 5 | 4 | 1 | 3 | 0 | 6.5 | 0 | ... |
| Basal ration + 6% no. 5 | 3 | 0 | 3 | 0 | 5.5 | 0 | ... |
| Basal ration + 3% no. 6 | 3 | 0 | 3 | 0 | 4.5 | 0 | ... |
| Totals or ave. | 28 | 6 | 21 | 1 | 6.1 | 3 | 28.4 |
| Basal ration + fish sol. + 3% no. 1 | 3 | 0 | 0 | 3 | 9.0 | 5.0 | 42.5 |
| Basal ration + fish sol. + 3% no. 2 | 3 | 1 | 0 | 2 | 3.0 | 2.5 | 30.0 |
| Basal ration + fish sol. + 6% no. 2 | 3 | 1 | 1 | 1 | 4.5 | 5.0 | 35.2 |
| Basal ration + fish sol. + 3% no. 3 | 4 | 0 | 0 | 4 | 7.5 | 5.5 | 35.7 |
| Basal ration + fish sol. + 3% no. 4 | 3 | 0 | 1 | 2 | 7.7 | 3.0 | 31.1 |
| Basal ration + fish sol. + 3% no. 5 | 4 | 1 | 1 | 2 | 6.3 | 4.5 | 49.2 |
| Basal ration + fish sol. + 6% no. 5 | 3 | 0 | 1 | 2 | 8.0 | 5.5 | 41.8 |
| Basal ration + fish sol. + 3% no. 6 | 3 | 0 | 1 [2] | 2 | 7.0 | 6.0 | 43.7 |
| | 26 | 3 | 5 | 18 | 6.9 | 4.7 | 39.6 |

[1] Litter size reduced to 6 pups per litter at 48 hours.

[2] Female died during parturition.

Eighteen out of 22 litters were weaned, and the average weight at weaning was 39.6 gm per pup, as compared to 28.4 gm per pup for the one litter weaned on yeast supplementation alone.

*Experiment 3*

Table 3 presents the data on the effects of the various test supplements with and without 5% fish solubles in the basal ration.

Since successful reproduction was obtained in the presence of both 3% and 6% brewers' yeast when fish solubles were added, it appeared to be a lack of reproduction factors in brewers' yeast rather than a toxic effect of the purines that caused the failure in reproduction. This was also indicated in the third experiment, where the results of supplementation with depurinized brewers' yeast were similar to those obtained with untreated brewers' yeast. Although fewer pups were lost in the first 48 hours, of those left alive with the mother at 48 hours only half survived through weaning. The weight of the pups at weaning, 27.2 gm per pup, also agreed closely with the value of approximately 29 gm (table 3, series 1) generally obtained on supplementation with purine containing brewers' yeast. Again, the addition of 5% fish solubles resulted in less sterility, larger litter size, heavier pups both at 48 hours and at weaning, and a greater per cent of pups weaned (table 3, series 2).

In an effort to determine whether or not vitamin $B_{12}$ could replace the reproduction and lactation factor or factors of fish solubles, a $B_{12}$ concentrate [4] was given as a supplement at 0.2%, an amount which furnished 5.5 μg of vitamin $B_{12}$ per 100 gm of ration. Another group was given vitamin $B_{12}$ by subcutaneous injection at a level of 2 μg once weekly. Due to limitations of supply at the time of this experiment, only 4 rats were used in the former and three in the latter assay. Both of these groups excelled all the others, in that there were no females sterile and no litters lost. In other respects

[4] Merck's, mix 3.

[3] Litter size reduced to 6 pups per litter at 48 hours.
[2] Female died during parturition.

TABLE 3

*Effects of brewers' yeasts, fresh liver, fish solubles or vitamin $B_{12}$ concentrate supplementation on reproduction and lactation in rats*

| DIET AND SUPPLEMENT | NO. OF FEMALES | | NO. OF LITTERS | | AVE. NO. OF PUPS PER LITTER | | AVE. WEANING WEIGHT PER RAT |
|---|---|---|---|---|---|---|---|
| | Bred | Sterile | Lost | Weaned | Born | Weaned [1] | |
| | | | | | | | gm |
| Series 1 | | | | | | | |
| Basal ration + 6% brewers' yeast | 10 | 0 | 6 | 4 | 5.3 | 4.5 | 29.4 |
| Basal ration + 10% liver + 6% brewers' yeasts | 7 | 14 | 2 | 4 | 4.7 | 3.8 | 44.1 |
| Series 2 | | | | | | | |
| Basal ration + 6% brewers' yeast | 28 | 21 | 21 | 1 | 6.1 | 3.0 | 28.4 |
| Basal ration + 5% fish solubles + 6% brewers' yeasts | 26 | 15 | 4 | 18 | 6.9 | 4.7 | 39.6 |
| Series 3 | | | | | | | |
| Basal ration + 6% brewers' yeast | 13 | 23 | 6 | 4 | 6.0 | 5.3 | 29.6 |
| Basal ration + 5% fish solubles + 6% brewers' yeast | 13 | 8 | 2 | 10 | 8.3 | 4.8 | 40.3 |
| Basal ration + 0.2% vitamin $B_{12}$ concentrate | 4 | 0 | 0 | 4 | 8.0 | 4.3 | 48.7 |
| Basal ration + 0.2% $B_{12}$ conc. + 5% fish solubles | 3 | 0 | 0 | 3 | 9.0 | 5.0 | 40.5 |
| Basal ration + vitamin $B_{12}$ by injection | 3 | 0 | 0 | 3 | 9.7 | 4.0 | 39.1 |
| Basal ration + $B_{12}$ injection + 5% fish solubles | 2 | 0 | 0 | 2 | 10.5 | 5.5 | 41.5 |

[1] Litter size reduced to 6 pups per litter at 48 hours.

the results with these groups compared favorably with the values obtained with 5% fish solubles. Addition of 5% fish solubles to the $B_{12}$-supplemented groups caused a further increase in the number of pups born and weaned per litter but not in the average weight per rat at weaning, which remained at about 41 gm per weanling rat (table 3, series 3).

The weight of the mother rats at the time of weaning is not recorded in the tables in this paper because the females were not found to lose weight during lactation.

Reproduction and lactation were good when the ration was supplemented with 5% fish solubles, which at this level is estimated to supply between 2 and 5 μg of vitamin $B_{12}$ per 100 gm of ration. Since vitamin $B_{12}$ concentrate ingested at 5.5 μg per 100 gm of ration similarly gave good results, it appears that the reproduction and lactation factor in fish solubles may well be vitamin $B_{12}$.

Previous studies by Ross ('42), Cunha ('44), and Spitzer and Phillips ('46) with the same or very similar yellow corn-soybean oil meal-alfalfa rations have shown either some, generally slight, improvement, or no improvement at all in the reproduction and lactation of rats when brewers' yeast has been used as a supplement. Van Landingham and Lyon ('47) reported similar slight improvement on the addition of brewers' yeast to a ration containing whole grain cereals, corn, wheat, oats, and solvent-extracted soybean oil meal supplemented with bone meal and certain vitamins.

The slight improvement may reflect the storage of factors by the particular test animals or it may indicate a borderline content of the factor or factors in brewers' yeast. It would seem that in general vitamin $B_{12}$ was not present in brewers' yeast in amounts adequate for reproduction and lactation. However, if strains of brewers' yeast should be developed which contain the order of magnitude of 1 μg of vitamin $B_{12}$ per gram of dried brewers' yeast, these strains would be an effective supplement to a natural plant diet for reproduction and lactation.

injection + 5% fish solubles 2 0 2 10.5 5.5 41.5

[1] Litter size reduced to 6 pups per litter at 48 hours.

SUMMARY

Nine brewers' yeasts, including one depurinated yeast, tested as supplements to a basal yellow corn-soybean oil meal-alfalfa ration for rats, failed to furnish the factor or factors needed for successful reproduction and lactation. Fresh beef liver at 5 to 10% of the ration and 5% fish solubles furnished the required factor or factors. In the relatively few animals used, vitamin $B_{12}$ fed at a level similar to that of the $B_{12}$ content of 5% fish solubles, or injected subcutaneously at one-half the oral dose, supported reproduction and lactation performances in rats equal to those given by 5% fish solubles under the conditions of these experiments. This suggests that the reproduction and lactation factor in fish solubles may well be vitamin $B_{12}$.

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# THE LYSINE REQUIREMENT FOR THE GROWTH OF SWINE

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## INTRODUCTION

The dietary significance of the amino acids for swine has been recognized for a considerable time, even though requirements for certain amino acids have been defined only recently. The first in a series of studies at this Experiment Station on the amino acid requirements of swine was reported by Bell et al. ('50).

Mertz et al. ('49) have recently shown lysine to be indispensable for growing pigs. Since most swine rations consist largely of cereal grains, which are generaly low in lysine, a knowledge of the quantitative requirement of swine for this amino acid is important.

The percentage of total protein in a diet influences the amount of lysine necessary for the maximum growth permitted by the level of protein. Grau ('48) has demonstrated this influence with chicks by determining their lysine requirement at several levels of protein. Brinegar et al. ('49, '50) reported observations on the lysine requirement of growing pigs fed diets containing 10.6% protein supplied by linseed meal or approximately 22% of protein supplied by either sesame meal or meat scraps, zein and wheat. These observations are presented in greater detail in the present report.

## LYSINE STUDIES WITH PIGS RECEIVING 10.6% DIETARY PROTEIN

### *Procedure*

Chester White pigs were placed on a commercial swine ration for 5 days following weaning and prior to individual

TABLE 1

*Composition of basal diets*

| CONSTITUENTS | DIET WITH PROTEIN CONTENT OF | | |
|---|---|---|---|
| | A 9.85% | B 21.1% | C 21.3% |
| | % | % | % |
| Linseed meal | 28.37 | | |
| Sesame meal | | 45.45 | |
| Meat and bone scraps | | | 15.13 |
| Zein | | | 4.20 |
| Wheat | | | 70.86 |
| DL-Methionine | 0.30 | 0.40 | 0.50 |
| L-Histidine monohydrochloride | 0.10 | 0.13 | 0.24 |
| DL-Tryptophan | | | 0.10 |
| Starch | 27.81 | 24.02 | |
| Glucose (cerelose)[1] | 25.00 | 23.00 | 1.00 |
| Ruffex[2] | 8.00 | 1.31 | 2.19 |
| Mineral salts[3] | 6.00 | 1.33 | 1.30 |
| Lard | 3.40 | 1.36 | 2.52 |
| Liver fraction "L"[4] | | 1.00 | 1.00 |
| Vitamins[5] | + | + | + |

[1] In the formation of the experimental diets, L-lysine monohydrochloride and cerelose (and glycine in the case of diet A) were added to bring the total ingredients to 100%.

[2] A commercial rice hull preparation by the Fisher Scientific Co.

[3] For diet A, see Bell et al. ('50). For diets B and C respectively, the following ingredients were included in the mineral mixture: NaCl, 90.6% and 98.0%; MgO, 7.54% and none; $FeSO_4 \cdot 7H_2O$, 1.48% and 1.6%; KI, 0.1% and 0.1%; $MnSO_4 \cdot 5H_2O$, 0.09% and 0.1%; $CuSO_4 \cdot 5H_2O$, 0.09% and 0.1%; $CoCl_2$, 0.05% and 0.05%; and $ZnCl_2$, 0.05% and 0.05%.

[4] Obtained from the Wilson Laboratories, Chicago, Ill.

[5] The following vitamins were added to each kilogram of basal mixture: Thiamine hydrochloride, 2.1 mg; riboflavin, 3.1 mg; niacin, 10.3 mg; calcium pantothenate, 14.8 mg; pyridoxine hydrochloride, 2.4 mg; folic acid supplement ("Folvite," supplied by Lederle Laboratories), 0.6 mg; choline chloride, 1.0 gm; vitamin A, 2,200 I.U. and vitamin D, 220 I.U., both supplied by a concentrated fish liver oil; mixed tocopherols, 0.2 gm.

feeding of their experimental diets. A period of 5 more days was allowed for adjusting them to their respective rations.

The basal diet (table 1, diet A) included linseed meal supplemented with methionine and histidine. These amino acids were added to the basal diet since a comparison of their content in linseed meal to that of proteins having a higher biological value for rats seemed to favor their inclusion (Block and Mitchell, '46–'47). Various levels of L-lysine monohydrochloride were added to this basal mixture containing 9.85% protein to give 5 experimental diets of differing lysine content. Glycine was then added to make each diet contain 10.6% protein ($N \times 6.25$).

Growth response to the diets over a 4-week period was used as a measure for comparing the levels of lysine. Daily feed intakes were periodically equalized at approximately 5% of body weight. Nitrogen balance data were obtained in order to permit a more critical evaluation of the growth response. Fecal and urine collections were made over a 5-day period following a preliminary period of at least 10 days.

*Results*

The data reported in table 2 clearly show that basal mixture A is deficient in lysine. Increasing the level of lysine in the diet caused corresponding increases in the average daily gain of the pigs. Since the initial weights of the pigs on the various levels of lysine were not identical, a more accurate measure for comparing the growth rates is obtained by expressing them as percentages of the initial weights rather than as units of gain. On this basis the daily rate of gain increased only up to the 0.58% level of lysine. The further small increase in the growth rate at the 0.74% level of lysine, if significant, appears to be accounted for by the larger feed intake of those pigs. The gain in weight per kilogram of feed consumed also increases with each increase in the level of lysine up to 0.58% of the diet, but is not further improved by additional lysine. The data for the apparently digested nitrogen retained in the body show that the increased growth of

the pigs receiving supplements of lysine involved storage in the form of protein tissue. From these data it appears that the minimum level of lysine necessary for the maximum growth and feed efficiency of weanling pigs fed a diet containing 10.6% protein is approximately 0.6% of the ration.

TABLE 2

*Average data for pigs receiving various levels of lysine in the linseed meal diet A*

| | TOTAL L-LYSINE IN DIET (%)[1] | | | | |
|---|---|---|---|---|---|
| | 0.34 | 0.42 | 0.50 | 0.58 | 0.74 |
| Number of pigs | 3 | 3 | 3 | 3 | 2 |
| Initial weight (kg) | 15.6 | 13.9 | 14.9 | 15.8 | 18.1 |
| Daily feed intake (% of body weight) | 4.67 | 4.64 | 4.54 | 4.63 | 4.71 |
| Daily gain (gm) | 245 | 252 | 289 | 356 | 420 |
| Daily gain (% of initial weight) | 1.57 | 1.81 | 1.94 | 2.25 | 2.32 |
| Grams gained per kilogram of feed | 276 | 311 | 336 | 370 | 372 |
| Apparently digested nitrogen retained (%) | 35.9 | 34.1 | 45.7 | 60.0 | 59.4 |

[1] The lysine content of the basal diet was determined by microbiological analysis of an acid hydrolysate of the protein, using *Leuconostoc mesenteroides* (P-60, 8042). Additions of L-lysine monohydrochloride were corrected to pure L-lysine.

## LYSINE STUDIES WITH PIGS RECEIVING APPROXIMATELY 22% DIETARY PROTEIN

### *Procedure*

Weanling Yorkshire pigs were used for these studies. The basal diets (table 1) included sesame meal (diet B) and meat and bone scraps, zein, and wheat (diet C) as the basal sources of amino acids. These proteins were substituted for linseed meal because of the purgative effect of the latter when fed at high levels. Methionine and histidine were included in diets B and C, and, in addition, tryptophan was included in diet C, since these proteins appeared to be low in the particular amino acids added (Block and Mitchell, '46–'47). The protein contents ranged from 21.1% to 22.1% in diet B and from 21.3% to 21.7% in diet C after lysine supplementation. Liver powder was included in the diets fed all but 10 of the pigs on diet B. This preparation was added in order to prevent a possible

lack of those nutrients which may not have been present in this diet. The 10 pigs which did not receive this supplement were equally distributed among all but the highest and lowest levels of lysine. Inspection of the individual data failed to reveal apparent differences in performance of the pigs due to omission of the liver powder.

The true digestibility of the lysine in basal diets B and C was determined by analyzing fecal excretions. Corrections for metabolic fecal fractions were obtained by analyzing fecal

TABLE 3

*Average data for the pigs receiving various levels of lysine in sesame meal diet B*

| | TOTAL L-LYSINE IN DIET (%)[1] | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0.57 | 0.75 | 0.97 | 1.07 | 1.17 | 1.32 | 1.63 |
| Number of pigs | 3 | 2 | 6 | 6 | 6 | 6 | 5 |
| Initial weight (kg) | 17.1 | 17.3 | 17.8 | 18.9 | 17.8 | 17.2 | 18.1 |
| Daily feed intake (% of body weight) | 4.41 | 4.50 | 4.34 | 4.30 | 4.34 | 4.34 | 4.26 |
| Daily gain (gm) | 336 | 388 | 431 | 486 | 483 | 464 | 492 |
| Daily gain (% of initial weight) | 1.96 | 2.24 | 2.42 | 2.57 | 2.71 | 2.70 | 2.72 |
| Grams gained per kilogram of feed | 349 | 379 | 416 | 439 | 453 | 451 | 462 |

[1] See footnote, table 2.

excretions from a protein-free diet which consisted of cerelose 25%, starch 60%, roughage [1] 4%, lard 5%, mineral salts 6% (Bell, '50) and vitamins (see table 1).

## *Results*

The data presented in table 3 show that the lysine requirement for weanling pigs receiving approximately 22% sesame protein is greater than the requirement determined when only 10.6% linseed protein was included in the ration. The average daily gain and feed efficiency were both improved as the lysine level was increased to 1.17% of the ration. Further

[1] Ruffex.

lysine supplementation to levels as high as 1.63% failed to improve the ration significantly.

The effect of adding lysine to the diet containing meat scraps, zein and wheat (diet B, 22% protein) is shown in table 4. The results are in agreement with those obtained by supplementing the 22% sesame protein diet with lysine. The rate and efficiency of gains made by the pigs receiving the rations containing 1.2% and 1.4% lysine were both higher than those of the pigs on lower levels of lysine. The 1.4% lysine level, however, failed to produce significantly better results

TABLE 4

*Average data for pigs receiving various levels of lysine in the meat scrap – zein – wheat diet C*

| | TOTAL L-LYSINE IN DIET (%)[1] | | | |
|---|---|---|---|---|
| | 0.96 | 1.00 | 1.20 | 1.40 |
| Number of pigs | 5 | 5 | 5 | 5 |
| Initial weight (kg) | 19.5 | 19.8 | 19.1 | 19.2 |
| Daily feed intake (% of body weight) | 4.56 | 4.55 | 4.50 | 4.48 |
| Daily gain (gm) | 446 | 465 | 499 | 507 |
| Daily gain (% of initial weight) | 2.29 | 2.35 | 2.61 | 2.64 |
| Grams gained per kilogram of feed | 380 | 388 | 425 | 430 |

[1] See footnote, table 2.

than 1.2%. The results of the two experiments (tables 3 and 4) show that the lysine requirement of weanling pigs receiving 22% protein is approximately 1.2% of the ration.

The averages of the data for the true digestibility of lysine for 6 pigs receiving basal diet B (without liver powder) and for 5 pigs receiving basal diet C were 88% and 90%, respectively. Application of these digestion coefficients to the lysine content of the basal rations places the requirement on a basis which takes account of digestibility. In such a case the requirement obtained from the trials including diets B and C was 1.1% of digestible lysine in the ration.

## DISCUSSION

The amino acid composition of the basal diets, shown in table 5, indicates that a difference in the proportion of amino acids in the rations was probably not the reason for a higher lysine requirement when 22% protein was fed as compared to the requirement when only 10.6% protein was included in the ration. Definite conclusions cannot be drawn from these

TABLE 5

*Amino acid content of the basal diets*

| | GRAMS AMINO ACID PER 16 GM NITROGEN [1] | | |
|---|---|---|---|
| | Diet A 9.85% protein | Diet B 21.1% protein | Diet C 21.3% protein |
| Arginine | 8.4 | 10.8 | 5.2 |
| Histidine | 2.6 | 2.8 | 2.8 |
| Lysine [2] | 3.4 | 2.7 | 4.5 |
| Tryptophan | 1.1 | 1.1 | 1.4 |
| Phenylalanine | 4.1 | 4.3 | 4.5 |
| Methionine | 4.1 | 3.8 | 3.4 |
| Threonine | 3.3 | 3.4 | 2.8 |
| Leucine | 5.5 | 5.8 | 10.6 |
| Isoleucine | 3.8 | 3.4 | 3.9 |
| Valine | 4.6 | 4.7 | 4.6 |

[1] Determined by microbiological techniques to be published in detail later and referred to by Loosli et al. ('49), with the exception that *Streptococcus faecalis* 9790 was used for the assay of isoleucine in sesame meal, meat scraps, zein, wheat and liver powder. Includes histidine, methionine and tryptophan added to the basal diets (table 1).

[2] Before supplementation with L-lysine monohydrochloride.

values, however, because both qualitative and quantitative information is lacking as to the essential nature of amino acids so far as swine are concerned.

The increased growth rate of the pigs receiving 22% protein over those receiving only 10.6% protein undoubtedly accounted for part of the increase in the lysine requirement. In addition, there was a decrease in the efficiency of utilization of the lysine consumed at the higher protein level. For example, the gain in weight per gram of lysine consumed was

64 gm for the pigs fed the 10.6% protein diet (0.58% lysine) and only 39 gm and 35 gm for those fed the 22% protein diets A (1.17% lysine) and B (1.2% lysine), respectively.

In evaluating these requirements individually there are several points which should be considered. The biological value of the proteins in the diets supplemented with the required amounts of lysine and the caloric value of the diets probably influenced the lysine requirements which were obtained.

These experiments show, as Grau ('48) previously reported, that any statements regarding the lysine requirement expressed as a percentage of the diet should be qualified in terms of the level of dietary protein. If the requirements determined by using the low and high protein rations for swine are expressed as percentages of the dietary protein, the difference between them is largely eliminated. The lysine requirement of 0.6% of the ration at the 10.6% level of protein is equivalent to 5.7% of the dietary protein, while the requirement of 1.2% at 22% protein corresponds to 5.5% of the dietary protein. This relationship of the lysine requirement to the level of dietary protein is in line with the concept stated by Almquist ('47), ". . . that the requirement of any indispensable amino acid for any rate of growth has a fixed proportion to the others in the diet." Since the recommended protein level for swine rations is variable, depending on the size of the animals, amino acid requirements are best stated as percentages of the dietary protein. Such expressions result in more consistent values than if the requirements are stated as percentages of the diet. The digestible lysine requirement of 1.1% of the 22% protein ration is equivalent to 5.0% digestible lysine in the dietary protein.

Analyses at this laboratory of the ingredients in typical swine rations, such as those containing 22% protein contributed by meat scraps or fish meal, soybean oil meal, dehydrated alfalfa meal, and corn, show these rations to contain from 1.2% to 1.4% of total lysine. These results show that lack of lysine is not likely to limit the growth of pigs fed the proper

combination of natural feedstuffs. Since, however, the lysine content of the above feed mixture is near the minimum requirement, care must be taken to balance swine rations with respect to lysine.

It is of interest to note that the requirement by swine of 1.2% lysine in the ration is rather close to a chick requirement of 0.9% (Almquist and Mecchi, '42; Grau, '48) and a rat requirement of 1.0% (Rose, '37).

## SUMMARY

Weanling pigs were fed rations containing: (A) 10.6% linseed meal protein supplemented with methionine and histidine; or (B) approximately 22% sesame meal protein supplemented with methionine and histidine; or (C) approximately 22% of a mixture of meat scraps, zein and wheat proteins which included added methionine, histidine and tryptophan. The effects of adding various levels of lysine to the basal mixtures were studied over a 4-week period.

The minimum level of lysine which produced the maximum gain in weight and feed efficiency was considered as the lysine requirement. For ration A, containing 10.6% protein, the lysine requirement was 0.6% of the ration. When rations were fed which contained approximately 22% protein the lysine requirement increased to 1.2% of the ration. The difference in these requirements is largely eliminated if they are expressed in terms of their proportion to the protein in the rations. The lysine requirements of 0.6% and 1.2% of the ration correspond to 5.7% and 5.5% of the protein in the 10.6% and 22% protein rations, respectively. The digestibility of the lysine in basal rations B and C was determined and applied to the requirement of 1.2% of the 22% protein ration. The digestible lysine requirement for weanling pigs fed 22% protein then became 1.1% of the ration, or 5.0% of the protein in the ration.

The weight range obtaining in the growth period to which these requirements apply was approximately 16 kg to 33 kg. The average daily gains of the pigs receiving optimum levels

of lysine were 382 gm for those receiving 10.6% dietary protein and 488 gm for those fed the 22% protein diets.

ACKNOWLEDGMENTS

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# PROCEEDINGS OF THE FOURTEENTH ANNUAL MEETING OF THE AMERICAN INSTITUTE OF NUTRITION

THE HOTEL TRAYMORE AND CONVENTION HALL, ATLANTIC CITY, NEW JERSEY, APRIL 17–21, 1950

## COUNCIL MEETINGS

Council Meetings were held in the Hotel Traymore on Sunday and Monday, April 16 and 17. Formal actions of the Council are reported in the minutes of the business meetings.

## SCIENTIFIC SESSIONS

The scientific program consisted of 7 half-day sessions at which 82 submitted papers were presented and a symposium on the "Nutritional Appraisal of Human Subjects" was held on the afternoon of April 20. Five papers were read by title. The Institute of Nutrition also participated in the Joint Session of the Federation on the evening of April 18.

## BUSINESS SESSIONS

Two business meetings were held: one at 4:30 P.M., Tuesday, April 18 and one at 4:30 P.M., Thursday, April 20. Items of business received attention as follows:

*Tuesday, April 18, 4:30* P.M. The meeting was called to order by President C. G. King. The President appointed Dr. James Waddell and Dr. A. G. Hogan to serve as a Tellers' Committee and ballots on the election of officers were transmitted to this Committee by the Secretary.

The report of the Treasurer, N. R. Ellis, was submitted. A report by the Auditing Committee, consisting of Dr. Hazel K. Stiebeling and Dr. E. M. Nelson, was presented by Dr. Stiebeling. The Auditing Committee reported that "receipts and

disbursements are properly recorded and that the Treasurer's report of April 4 is substantiated by the records."

The annual report of the Editorial Board of THE JOURNAL OF NUTRITION was submitted by the Editor, Dr. George R. Cowgill. The report follows:

### *Annual Report of the Editor of The Journal of Nutrition*

This annual report covers the volumes of THE JOURNAL OF NUTRITION published during 1949 as well as general matters arising since the last annual meeting of the American Institute of Nutrition in Detroit, Michigan, April 18, 1949.

#### *Papers published and related data*

During 1949 there were 161 manuscripts offered for publication, compared with 168 for the previous year. A total of 34 were rejected for various reasons. These constituted 21% of the total submitted. This percentage of rejections is slightly below that for the previous year, namely 24%.

In accordance with the new arrangement made with our publisher, The Wistar Institute, three instead of two volumes were published during 1949, namely volumes 37, 38 and 39. They contained 43, 43 and 41 papers, respectively, making a total of 127 for the year. The average number of papers per issue was about the same as for 1948, being 10.8, 10.8 and 10.2 articles as compared with 10.7 and 11.1 for the two volumes of the previous year. The average number of pages per article for these volumes, respectively, proved to be 12.6, 12.4 and 14.5, figures slightly higher than the corresponding ones for the previous year, namely 11.6 and 11.9 pages.

#### *Picture on front cover*

Beginning with the January, 1949, issue a new picture appeared on the front cover, namely that of Stephen M. Babcock, which replaced one of Chittenden. The Babcock picture was used on all of the issues for 1949.

At the annual meeting of the Editorial Board in Detroit it was voted to have the picture changed with each volume, beginning with 1950, and, if possible, to use the same picture as a frontispiece for the volume and to run with it a short biography of the scientist in question. The Editorial Board also voted that pictures of F. G. Hopkins, Graham Lusk and C. Eijkman be the ones to be used with the three volumes to appear in 1950. The issues that have appeared in 1950

to date (January, February and March) have carried the Hopkins picture.

Suggestions of deceased scientists whose pictures might be used in this way are always welcomed by the editor.

### *Cumulative 10-year index*

As announced in our last report, a cumulative index was being prepared covering the second 10 years of life of THE JOURNAL OF NUTRITION. Copy for it was completed late in 1949, and it finally went to press early in 1950. It has now been distributed to its subscribers.

Under date of March 14, 1950, Wistar Institute reported on the cumulative index as follows:

| | | |
|---|---|---|
| Orders received direct (non-members of Am. Inst. Nutrition) | | 853 |
| Orders received direct *from* Am. Inst. Nutrition members | 87 | |
| Orders received direct through Am. Inst. Nutrition members | 33 | |
| | 120 | |
| Total Am. Inst. Nutrition orders | | 120 |
| Total orders received | | 973 |

"To date 632 copies have been mailed. The remaining 341 are awaiting payment from memorandum invoices which were mailed January 30th."

### *Personnel of editorial board*

At the last annual meeting three new members of the editorial board were elected to replace those whose terms had expired. The new members were William J. Darby, David M. Hegsted, and Pearl P. Swanson. It may be of some interest to the officers of the society as well as the members of the editorial board to know that the names of the board members printed on the front cover of each isssue are arranged in order of year of appointment, the first three being those of members serving their last year of membership, the next three those with two years to serve, the next three those with three years, and the last three those of the newest board members who are serving the first of their 4-year term. In each group of three the names are listed alphabetically. Thus, if one wishes to know which three members are due to retire next, he has merely to consult the list on the front cover of any current issue and examine the first three names.

*Editorial problems*

These continue to be the same as those encountered in previous years. An effort is always made to have an annual meeting of the editorial board at each federation meeting. At such meetings these editorial problems are reviewed, an exchange of ideas obtained, actions of the editor reviewed, et cetera. It is still a fact that the amount of editorial work needed on manuscripts would be greatly reduced if every author endeavored to prepare his manuscript in accordance with the instructions to contributors printed on the inside back cover of every issue. These instructions are also set forth in greater detail in the leaflet A GUIDE FOR AUTHORS — THE WISTAR INSTITUTE JOURNALS published by Wistar. Recently, in an effort to get more action along this line from future contributors, the editor asked Wistar Institute to mail a copy of this GUIDE to each member of the American Institute of Nutrition.

*General comments*

This is a fitting place to record the editor's appreciation of the services rendered by the individual members of the editorial board. During the summer months when it happened that quite a few members were not available to read manuscripts, other members did more than their share of this work. Later, when the board was "at its full strength," the editor made an effort to relieve the "summer readers" for a period by suitable and equitable distribution of manuscripts as they came in. All of the board members have been very helpful to the editor by their statements of frank reactions to papers and their concrete suggestions of what might be done with them in the interest of all parties concerned, authors and the Journal. For these services the editor is very grateful indeed, and thanks the editorial board.

The report of the Committee on Rules for Awards (H. E. Carter, R. R. Sealock, C. D. Tolle, J. Waddell and W. C. Russell, Chairman) was submitted by Dr. Russell. The report was adopted. This report follows:

*Rules governing awards of the American Institute of Nutrition*

For each award there shall be a Nominating Committee of three members appointed by the President of the Institute to receive and make nominations. It shall be the duty of a Committee to obtain from nominators, or from other sources, complete information about

the nominees, and, if it so desires, to make appropriately documented nominations of its own. To maintain a continuity of experience and to provide for rotation of membership, the first Committee, for each award, shall consist of one member appointed for a three-year term, one for a two-year term, and one for a one-year term. Thereafter members shall be appointed for three-year terms. For the first Committee, the member appointed for the one-year term shall serve as Chairman, and thereafter, the member with one year yet to serve shall hold this office.

For each award, there shall be a Jury of Award of 5 members to be named by the President in accordance with the provisions stated below. To provide for rotation of membership, and at the same time maintain a continuity of experience, the first Jury shall consist of two members appointed for three-year terms, two for two-year terms, and one for a one-year term. Thereafter all appointments shall be for three-year terms. After a Juror has served a term of any length, he shall not be eligible for another appointment until after the lapse of one year. The President of the Institute shall annually designate one member of the Jury as chairman. When a vacancy occurs on a Jury, the President shall fill the vacancy by appointment for the unexpired term. If a juror is nominated for an award, he shall resign his appointment immediately and the President shall fill the vacancy by an *ad interim* appointment. Each Jury of Award shall use the following procedure in receiving and considering nominations.

Promptly after January 1 of the year in which the award is to be made, chairmen of nominating committees shall transmit all documents to the President of the Institute, who in turn shall send them to the chairmen of the appropriate juries. If the name of an individual is before more than one jury, the President shall at the same time inform the chairmen of the juries concerned. The chairman of a jury will then transmit the nominations with supporting data to the members of the jury, requesting prompt review and compliance with the procedure described below.

A chairman shall first ask the members of a jury whether any candidate should be given consideration for the award. If a majority answers in the negative, no award will be made. If a majority answers in the affirmative, then the jury shall at once proceed to ballot, each member of the jury indicating to the chairman his first, second, and third choices. If a majority of the members of a jury select as first choice the same person from the list of nominees, such choice shall be final and that person shall be designated as the recipient of the award. If there is no such agreement, the chairman shall weigh each first choice as 3, each second choice as 2, and each third choice

as 1, and shall total the score of each nominee obtained by this method. He shall then prepare a list of preferably not more than 4 of those having the highest total scores. Balloting shall be continued on the candidates so selected. On each subsequent ballot each member of a jury shall vote, and the chairman shall eliminate the candidate having the lowest point score until but two remain. From these two, the jury shall select the recipient. However, if on any ballot any candidate is the choice of the majority of the members of a jury, he shall be declared elected.

Not later than February 15, the chairman of a jury shall report the decision of the jury to the President of the Institute, giving the number of affirmative and negative votes. At the same time he shall send to the President a summary of the scientific contributions on which the award was based.

If the vote is three to two in favor of the decision, the President shall refer the report to the Council of the Institute for consideration. If the Council does not approve, it must then make the final decision concerning the selection of a recipient not later than three weeks before the opening day of the annual meeting. The President shall excuse from balloting, however, any member of the Council who may be designated as a candidate for the award. If the decision of a jury has been reached by an affirmative vote of 4 or 5 members, the action need not be communicated to the Council, unless so required by the donor of the award or unless in the judgment of the President the decision is not in keeping with the intent of the award.

After awards have been made, each recipient shall be notified by the President of the Institute, who shall take steps to announce the awards in the public and scientific press at an appropriate time and not later than one week before the opening day of the annual meeting at which the awards are to be made.

### *Additional recommendations*

It is recommended that the conditions governing the award made available by the Nutrition Foundation, Inc., now known as the Osborne and Mendel Award, set forth in paragraphs one to 10, inclusive, of the Report of the Special Committee on Rules and Regulations Governing the Annual Award Made Available by the Nutrition Foundation, Inc., J. Nutrition, *35:* 736–737 (1948), be continued. It is further recommended that paragraphs 12 to 15, inclusive, of the above-mentioned report be revoked and that the rules of the American Institute of Nutrition governing awards apply to the Osborne and Mendel Award.

It is recommended that the membership of juries of award be anonymous.

It is recommended that the Secretary of the Institute supply each member of a nominating committee and of a jury upon appointment, and each member of the Council upon election, descriptions of the several awards, a copy of the Regulations Governing Awards, and a list of the previous recipients of the awards.

It is recommended that each of the awards under the jurisdiction of the Institute be announced in the September and October issues of the Journal.

The report of the Committee on Program Policy (G. R. Cowgill, F. S. Daft, R. M. Leverton, E. W. McHenry and W. H. Griffith, Chairman) was presented by Dr. Griffith. This report follows:

*Report of Committee on Program Policy*

The Constitution of the American Institute of Nutrition contains the following statement of its purposes: "The purposes of the society are to further the extension of the knowledge of nutrition and to facilitate personal contact between investigators in nutrition and closely related fields of interest." It may be assumed that the scientific program at annual meetings is intended to support the aims of the society. The aims are fulfilled in theory by a gathering together of interested workers who present and defend publicly the results of their studies with benefit to both the speaker and the listeners.

It is axiomatic that optimum benefits will not be obtained from a scientific program if too many papers are presented in too short a time, particularly as an otherwise interested and sympathetic audience is worn down by days of attendance. It is entirely proper, therefore, to review the program policy from time to time to determine if this part of the annual meeting is continuing to further the purposes of the society. This is not only proper but necessary in a growing society because there are limitations in the number of days that may be spent in attendance at a meeting, in the number of rooms that may be procured for meetings and in the number of minutes that may be allowed for individual presentations. The sharp limitation that now exists with respect to a suitable location for the meeting of the Federation is a pertinent illustration of the restrictive effect of an increase in the number of scientific workers who have a legitimate claim to a place on the programs of the affiliated societies. Presumably, this society can modify its program as it sees fit, but the members are still faced with the bewildering complexity of the Federation program as a whole.

The programs of the American Institute of Nutrition in the last 4 years may be summarized as follows:

a. No multiple sessions have been necessary.

b. Of the 8 possible half-day sessions, the numbers used by the society were 4, 7, 6 and 8, respectively.

c. These half-day programs have included papers transferred from other societies. Without the transfers, and on a basis of 11 to 12 papers per program, the half-days required were 4, 6, 5 and 7, respectively.

The current program includes 82 papers, exclusive of 5 read by title and 5 papers on the symposium. These 82 papers include 6 transferred from other societies. Two papers were transferred from Nutrition to other societies. Eight half-days will be used.

It is a fair conclusion that the number of papers submitted to the Secretary has not been in excess of the total time available in single half-day meetings for presentations and discussions limited to approximately 15 minutes per paper. This may not be true in the future and an appreciable increase may necessitate multiple sessions.

Suggestions for improvement have ranged from programs of selected and invited speakers to programs containing all papers that are submitted, regardless of multiplicity of simultaneous sessions. Many find the present programs entirely satisfactory.

The Committee has considered these problems. Agreement on specific measures has not been possible. A fundamental difficulty has been the problem of program arrangements that vary significantly from the procedures followed by the other societies of the Federation. We are in a period of growth. Growing pains are uncomfortable. It is not believed that the problem of the annual program of the Institute has reached a point which demands drastic action, action which may require restriction of papers and which may result in a retardation of the further development of the society. No change is recommended in the general program procedure.

It is recommended that a special statement on preparation of lantern slides be included in the preliminary announcement of the annual meeting. A recent publication of the American Chemical Society on this subject might well be the basis of recommendations concerning size of lettering, amount of material included, etc.

It is recognized appreciatively that an effort is now made to concentrate presentations on specific topics in the entire program of the Federation. The difficulties inherent in this respect are obvious. Further progress along this line is urged.

The report of the Committee on Program Policy was adopted. With reference to this report Dr. Griffith made the following motion:

"It is moved that the Secretary of the Institute of Nutrition be authorized to include in preliminary announcements of the annual meeting a pertinent statement on the preparation of proper lantern slides and that the affiliated societies of the Federation be urged to take similar action." This motion was adopted.

A progress report by Dr. Robert E. Johnson, Chairman of the Committee on Registry of Nutritional Pathology, which had been mailed to the Secretary, was read by the Secretary.

Dr. Walter C. Russell presented a report as a representative of the American Institute of Nutrition to the Division of Biology and Agriculture of the National Research Council. Dr. Russell also reported upon his work as a representative to the Food and Nutrition Board.

The following recommendations of the Council were approved:

1. That the annual dues for the year 1950–51 be $1.00.
2. That the Treasurer shall order a suspension of the subscription of a member to THE JOURNAL OF NUTRITION if dues are not paid by December 31 of the current year.

President King reported certain actions by the Federation Executive Committee. It was recommended by the Executive Committee that the annual dues of the Federation be $2.00. Members were requested to consider the question of whether or not the Federation should accumulate sums of money considerably in excess of probable current budget needs which might be used for the purchase of property to serve as a headquarters.

The report of Dr. A. J. Carlson to the Executive Committee on the activities of the National Society for Medical Research was discussed. Dr. King called attention to the action of the Executive Committee, which recommended that each Society mail to members a statement inviting voluntary contributions of $1.00 or more to the National Society for Medical Research.

The Tellers' Committee reported the election of the following officers for the year beginning July 1, 1950:

*President:*
WENDELL H. GRIFFITH
*Vice President:*
CLIVE M. MCCAY
*Treasurer:*
N. B. GUERRANT
*Councillor:*
WALTER C. RUSSELL

For Associate Editors of the Journal of Nutrition the Tellers reported that Carl V. Moore, Harold H. Williams and Esmond E.

Snell had been elected. President King announced that Dr. Esmond E. Snell would be unable to serve as Associate Editor and that the Council had recommended that Dr. Vernon H. Cheldelin be elected in place of Dr. Snell. It was voted to accept this recommendation of the Council.

The meeting was adjourned at 5:32 P.M.

*Thursday, April 20, 4:30* P.M. The second business meeting was called to order by President C. G. King.

The Tellers' Committee reported the results of the voting on suggestions for the Nominating Committee. The names of the 15 members receiving the highest number of votes were submitted for the guidance of the President.

It was voted to approve the Council's recommendations for election to membership. The following persons were elected to membership in the American Institute of Nutrition:

J. Alexander Campbell
James R. Couch
Tony J. Cunha
Benjamin H. Ershoff
Ernest Geiger
Robert P. Geyer
Elizabeth M. Hewston
James M. Hundley
Harry D. Kruse
Richard W. Luecke
Marjorie M. Nelson
Paavo K. Roine
Arnold E. Schaefer
Bernard S. Schweigert
Maurice E. Shils
Harry Spector
Rudolph M. Tomarelli
Anne W. Wertz
Alvin C. Wiese

Dr. George R. Cowgill discussed the needs for library materials of the Institute of Nutrition of Central America and Panama. Dr. Nevin S. Scrimshaw discussed the work of this Institute and its library needs and made available printed instructions for sending reprints to its library. The address of this Institute is as follows:

Instituto de Nutrición de Centro América y Panamá
Jardín Botánico
Guatemala, Guatemala, C. A.

Dr. Conrad A. Elvehjem presented the report of the Joint Commitee on Nomenclature. The report was adopted. This report is as follows:

*Report of Committee on Biochemical Nomenclature*

Dr. E. J. King, the managing editor of the Biochemical Journal, has informed Dr. R. J. Anderson that the British journal proposes to employ the symbol $Q_x$ to represent metabolic quotients exclusively in the units *microliters per milligram dry weight of biological material per hour.* Where the metabolite x is a solid or a liquid, it is to be conventionally regarded as a gas at normal temperature and pres-

sure, one micromole of x being equivalent to 22.4 $\mu$l. Where other units are employed, e.g., *micromoles per milligram per hour* or *microliters per milligram of nitrogen per second*, the italic capital $Q$ is to be replaced by a lower case italic $q$. In all cases, the units must be clearly defined at the first mention. Production or removal are to be represented by plus or minus signs, aerobic or anaerobic conditions by superscripts such as $O_2$, and $N_2$, and the presence of another gas phase in the same way; as, for example, by the superscript $H_2$. The substrate may be indicated, if necessary, in parentheses following the $Q$ symbol, e.g., $\frac{Q^{N_2}}{CO_2}$ (pyruvate).

The term "vitamin P" was first applied to a substance present in lemon juice. It was said to be effective in reducing the extent of hemorrhages and extending the duration of life in scorbutic guinea pigs and was also proposed for the treatment of vascular purpura. Subsequent studies have failed to substantiate these claims and the identity of a substance of a vitamin nature has not been established. Continued application of the term "vitamin P" to one or another of a group of polyphenolic substances will lead only to confusion. It is therefore recommended that the term "vitamin P" should no longer be employed.

There was further discussion of the desirability of promoting the activation of the Registry of Nutritional Pathology. Interest in this opportunity was expressed and the opinion was developed that the Committee in charge of this work should proceed actively during the coming year with the work of setting up this Registry.

The meeting was adjourned at 5:30 P.M.

## DINNER AND PRESENTATION OF AWARDS

The Institute held its annual dinner on Wednesday evening, April 19, in the Hotel Madison. The program consisted of the presentation of awards, with very stimulating introductions of, and responses by, the recipients. President C. G. King served as master of ceremonies.

The Mead Johnson Vitamin B-Complex Award was presented to Dr. William B. Castle, Harvard University Medical School, "In recognition of his classic investigations leading to the concept of intrinsic and extrinsic factors in pernicious anemia, and of his studies elucidating the relation of vitamin $B_{12}$ to this concept." A 10-minute presentation talk was given by Dr. Frederick J. Stare, in which he referred in a delightful and inspirational way to the academic life and scientific contributions of the awardee.

The Borden Award in Nutrition was granted to Professor H. C. Sherman, Columbia University, "In recognition of his research emphasizing the nutritive importance of components of dairy products." A 10-minute presentation talk was given by Dr. Grace MacLeod, Professor Emeritus, Teachers College, Columbia University, concerning Professor Sherman's remarkable record as an educator in the field of nutrition. Due to the critical illness of Mrs. Sherman, Professor Sherman could not be present, and his daughter, Dr. Caroline Sherman Lanford (Mrs. Oscar E. Lanford) received the award in his behalf.

The Osborne and Mendel Award was presented to Dr. Conrad Arnold Elvehjem, University of Wisconsin, in recognition of recent basic research regarded by the Institute as having great significance in the science of nutrition. The award was given "In recognition of his contributions to our knowledge of the vitamin and amino acid requirements of experimental animals." A 10-minute presentation talk was given by Dr. Henry A. Lardy, Department of Biochemistry, University of Wisconsin.

In each instance the award presentation was followed by an informal acceptance talk, about 5 minutes in length, by the awardee. These talks, in addition to the presentation addresses, provided a delightful part of the evening's program, and served as a real inspiration to the dinner guests.

## COMMITTEES FOR 1950–51

President C. G. King appointed the following committees for the year beginning July 1, 1950:

### *Nominating Committee*

W. J. Darby, Chairman
O. A. Bessey
A. G. Hogan
L. A. Maynard
A. H. Smith

### *Committee on Registry of Nutritional Pathology*

R. E. Johnson, Chairman
O. A. Bessey
P. L. Phillips
W. H. Sebrell, Jr.

### *Representatives on Joint Committee on Nomenclature*

H. J. Almquist
C. A. Elvehjem

*Representative to the Division of Biology and Agriculture, National Research Council*

Walter C. Russell

*Representative to the Food and Nutrition Board, National Research Council*

Walter C. Russell

*Representative to the 18th International Physiological Congress*

Floyd S. Daft

Respectfully submitted,
JOSEPH H. ROE, *Secretary*
American Institute of Nutrition

## MEAD JOHNSON AND COMPANY 'B-COMPLEX' AWARD

Nominations are solicited for the 1951 Award of $1000.00 established by Mead Johnson and Company to promote researches dealing with the B-complex vitamins. The recipient of this Award will be chosen by a Jury of Award of the American Institute of Nutrition and the formal presentation will be made at the annual meeting of the Institute in the spring of 1951.

The Award will be given to the laboratory or clinical research worker in the United States or Canada who, in the opinion of the judges, has published during the previous calendar year, January 1 to December 31, the most meritorious scientific report dealing with the field of the 'B-complex' vitamins. While the award will be given primarily for publication of specific papers, the judges are given considerable latitude in the exercise of their function. If in their judgment circumstances and justice so dictate, it may be recommended that the award be made to a worker for valuable contributions over an extended period but not necessarily representative of a given year. Membership in the American Institute of Nutrition is not a requisite of eligibility for the award.

To be considered by the Jury of Award, nominations for this award for work published in 1950 must be in the hands of the Chairman of the Nominating Committee by January 1, 1951. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate the task of the Jury of Award in its consideration of the nomination.

E. E. Snell
*Department of Biochemistry*
*University of Wisconsin*
*Madison 6, Wisconsin*

Chairman, Nominating Committee

## OSBORNE AND MENDEL AWARD

Nominations are invited for the Osborne and Mendel Award of $1000.00 established by the Nutrition Foundation, Inc., for the recognition of outstanding accomplishments in the general field of exploratory research in the science of nutrition. It shall be given to the investigator who, in the opinion of a Jury of Award, has made the most significant published contribution in the year preceding the annual meeting of the Institute, or who has published a series of contemporary papers of outstanding significance.

The Award will be presented at the annual meeting of the American Institute of Nutrition.

The recipient will be chosen by a Jury of Award of the American Institute of Nutrition. As a general policy, the Award will be made to one person. If, in the judgment of the Jury of Award, an injustice would otherwise be done, it may be divided among two or more persons. Normally preference will be given to research workers in the United States and Canada, but investigators in other countries, especially those sojourning in the United States or Canada for a period of time, are not excluded from consideration. Membership in the Institute of Nutrition is not a requirement for eligibility and there is no limitation as to age.

Nominations may be made by anyone. Nominations for the 1951 Award, accompanied by data relative to the accomplishments of the nominee, must be sent to the Chairman of the Nominating Committee before January 1, 1951.

ROBERT V. BOUCHER
*Pennsylvania State College*
*State College, Pennsylvania*

CHAIRMAN, NOMINATING COMMITTEE

## BORDEN AWARD IN NUTRITION

Nominations are solicited for the 1951 Award of $1000.00 and a gold medal made available by the Borden Company Foundation, Inc. The American Institute of Nutrition will make this award in recognition of distinctive research by investigators in the United States and Canada which has emphasized the nutritive significance of the components of milk or of dairy products. The award will be made primarily for the publication of specific papers, but the Jury of Award may recommend that it be given for important contributions over an extended period of time. The award may be divided between two or more investigators. Employees of the Borden Company are not eligible for this honor.

The formal presentation will be made at the annual meeting of the Institute in the spring of 1951. To be considered for the award, nominations must be in the hands of the Chairman of the Nominating Committee by January 1, 1951. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate consideration for the award.

HAZEL K. STIEBELING
*Bureau of Human Nutrition and Home Economics*
*U. S. Department of Agriculture*
*Washington, D. C.*

CHAIRMAN, NOMINATING COMMITTEE

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